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CYTOLOGY OF THE HUMAN ADENOHYPOPHYSIS AS RELATED TO BIOASSAYS FOR TROPIC HORMONES*

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ONE of the major problems confronting any pathologist is the interpretation of cellular structure in terms of function. Although the microscopic structure of the human hypophysis has been known for nearly a hundred years, such functions as have been assigned to the various cell types have been deduced indirectly by correlating hypophyseal histology with the appearance of other endocrine glands. Recent experiments pointing to the important role of the hypophysis in the induction of malignant tumors in animals (1) and the clinical observation that hormone therapy may produce striking, if temporary, remissions in some human cancers would seem to justify a study of the human hypophysis by methods as precise as have previously been applied to the glands of rats and mice. It occurred to us that it might be helpful to perform bioassays on individual human hypophyses, one half of which could also be studied histologically.

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MATERIALS AND METHODS

The hypophysis was removed at autopsy from 18 patients, 9 male and 9 female. No attempt at selection of patients was made, as we hoped to obtain hypophyses with the wide range of structure commonly seen in routine autopsies. It should be emphasized that none of the patients can be considered "normal" in the sense that they met sudden accidental death; all had been subjected to the stress of a fatal illness for a more or less prolonged period. Several patients had received hormone therapy for a variety of reasons, and some had endocrinologic disorders. The pertinent clinical and anatomic data on these patients are summarized in Tables 1 and 2.

All bodies were kept in a refrigerator until the hypophyses could be removed. Following removal each gland was weighed immediately. The anterior lobe was dissected free of the posterior and then divided in the midsagittal plane. One half was frozen in dry ice for bioassay while the other was fixed in a mixture of one part 40 per cent formaldehyde and nine parts saturated mercuric chloride for microscopic study.

The bioassays were performed in triplicate in 26-day old hypophysectomized rats. All hypophysectomies were performed via the parapharyngeal approach. Starting one week after hypophysectomy, the test rats were injected for four days with a total daily aliquot of 7.5 mg. of adenohypophyseal homogenate given in divided doses. Twenty-four hours after the last injection, the rats were killed and the sellae examined for completeness of hypophysectomy. The endocrines were then removed and weighed to the nearest tenth of a milligram on a Roller-Smith torsion balance. A note was made as to the opening of the vagina and the presence of follicles and corpora lutea in the ovaries of female rats.

The half of each hypophysis that was used for histologic study was further divided sagittally into three blocks of equal thickness. Sections from each block were stained with the periodic-acid Schiff (PAS) technique, using an Orange G counterstain, with acid hematein, with hematoxylin and eosin, with chromotrope-aniline blue (2) and with the modified Mallory aniline blue technique.

A differential count was performed on every fifth field of one section from each of the three blocks which had been stained with PAS-Orange G. This method was chosen for quantitative studies rather than the conventional Mallory aniline blue technique because of recent histochemical work relating the Schiff positive cells ("basophils") to secretion of the gonadotropins and thyrotropin—all of which hormones contain glycoprotein (3–7)—and to ACTH (7–9).

With PAS technique, the following cell types can be differentiated:

1. *Normal basophils* (*maximal mucoid cells* (6)): These are large, round or ovoid, Schiff positive, densely granular cells which occur chiefly around the periphery of the anterior lobe in a normal hypophysis (Fig. 1).

2. *Hyaline basophils* (*Crooke's cells* (10)): These cells, which appear to be derived from normal basophils, contain areas of pale, hyaline cytoplasm which is Schiff negative and stains a clear sky blue with trichrome techniques. They may contain a few Schiff and hematoxylin positive granules forming a halo near the nucleus or around the periphery of the cell.

3. *Amphophils* (*sparsely granulated basophils* (11), *intermediate mucoid cells* (6)): These are rounded or polygonal cells which are frequently larger than the normal basophils and contain sparser granules which are often irregularly distributed and may vary in size (Figs. 1 and 2). The granules take PAS and hematoxylin like those of the normal

TABLE 1. SUMMARY OF CLINICAL AND ANATOMIC DATA ON MALE PATIENTS

Case	Age (yrs.)	Diagnosis	Thyroid	Adrenals	Testis	Prostate	Therapy	Gonad.* M - F	TSH M - F	ACTH M - F
1	19	ruptured cerebral aneurysm	8 Gm., focal activity	thin cortices with small nodules	active spermatogenesis	normal size, high epithelium		++ - ++	0 - +	0 - 0
2	50	malignant melanoma, metastases	28 Gm., large follicles, low epithelium	bilateral metastases	hypospermatogenesis	fibrosis, cuboidal epithelium	triethylene phosphoramide, 0.5 cc./day for 18 days	+ - tr	0 - 0	0 - 0
3	63	myelogenous leukemia, alcoholic cirrhosis	20 Gm., large follicles, low epithelium	22 Gm., lipoid depletion	hypospermatogenesis, tubular fibrosis	fibrosis, squamous metaplasia, flat epithelium	cortisone, 100 mg. /day for 7 days	+ - tr	0 - 0	0 - 0
4	66	lymphatic leukemia, cerebrovascular accident	25 Gm., large follicles, flat epithelium	25 Gm., cortical nodules	active spermatogenesis	nodular, sl. enlarged, high epithelium		++ - ++	0 - 0	+ - +
5	71	coronary sclerosis, congestive failure	18.5 Gm., focal activity	15 Gm., abundant lipoid	active spermatogenesis, focal tubular atrophy	invasive carcinoma (microscopic)		+ - +	0 - +	0 - 0
6	72	ca. of larynx, bronchopneumonia	20 Gm., flat epithelium	17.5 Gm., cortical nodules	total atrophy, tubular fibrosis	small with flat epithelium		+ - +	0 - 0	0 - 0
7	73	ca. of colon, metastases	19 Gm., flat epithelium	19 Gm., cortical nodules	hypospermatogenesis	benign hypertrophy		+ - +	0 - 0	0 - 0
8	80	pemphigus	17 Gm., cuboidal epithelium	26 Gm., thick nodular cortices	aspermatogenesis, ++ Sertoli cells	benign hypertrophy	cortisone, 300 mg. /day for 5 days; ACTH, 20 mg./day for 9 days	0 - tr	0 - +	0 - 0
9	82	coronary thrombosis	thyroiditis, fibrosis	10 Gm., thick cortices	hypospermatogenesis	removed 6 days, benign hypertrophy		++ - ++	0 - 0	0 - 0

* Stored gonadotropins in hypophysis assayed in male (M) and female (F) rats.

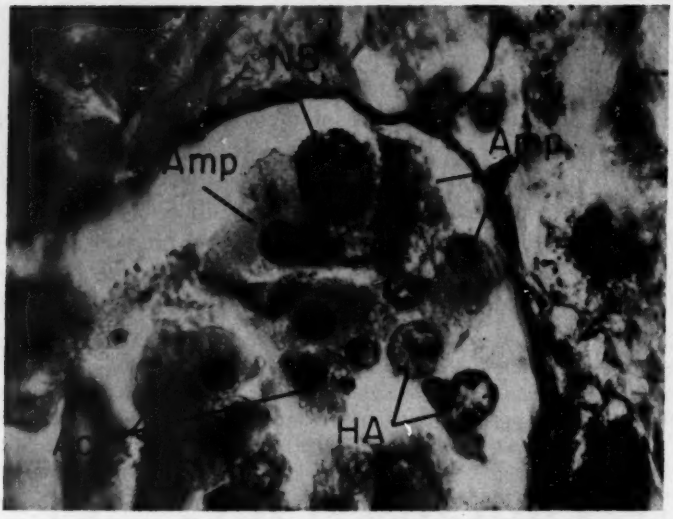

FIG. 1. Hypophysis in Patient 15, a 64-year-old female with coronary thrombosis. **NB** is an intensely Schiff positive normal basophil; **Amp** a weakly Schiff positive amphophil; **Ac** an acidophil; and **HA** a hypertrophic amphophil. High gonadotropins; no TSH or ACTH. (Periodic-acid Schiff-Orange G, ×750.)

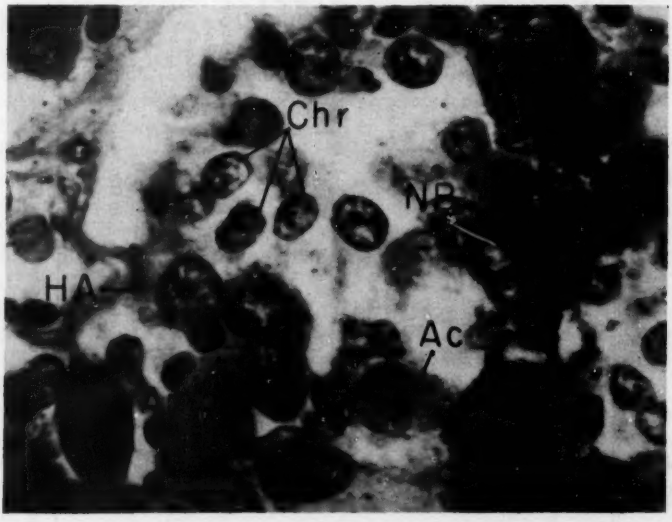

FIG. 2. Hypophysis in Patient 6, a 72-year-old male with carcinoma of the larynx. Contrast the very large vesicular hypertrophic amphophil containing a prominent nucleolus (**HA**) with smaller chromophobes (**Chr**) surrounding it. Moderate gonadotropins; questionable TSH; no ACTH. (Periodic-acid Schiff-Orange G, ×1,000.)

TABLE 2. SUMMARY OF CLINICAL AND ANATOMIC DATA ON FEMALE PATIENTS

Case	Age (yrs.)	p/g*	Diagnosis	Thyroid	Adrenals	Ovary	Endometrium	Breast	Therapy	Gonad.† M - F	TSH M - F	ACTH M - F
10	30	0/0	brain tumor	small follicles, low epithelium	15 Gm., thin cortices	large corpus luteum	premenstrual	chronic cystic mastitis		++ - ++	0 - 0	0 - 0
11	32	1/?	malignant hypertension, sympathectomy	25 Gm., large follicles, flat epithelium	20 Gm.	dense stroma, follicle cysts	atrophic	normal		+ - +	0 - +	0 - 0
12	54	2/3	ca. of breast, metastases	18 Gm., small follicles, fibrosis	coarse lipid vacuoles	atrophic	atrophic	ca.; chr. cystic mastitis	testololactone, 150 mg./wk. for 4 wks.; testosterone, 300 mg./wk. for 19 wks.; estinyl, 4 mg./day for 15 days	tr - tr	0 - 0	0 - 0
13	62	?	ca. of cervix, pelvic exenteration	9 Gm., colloid nodules, low epithelium	16.5 Gm., 1-cm. cortical adenoma	surgically absent 16 years		no data		++ - ++	0 - 0	0 - 0
14	63	4/11	diabetes, mesenteric thrombosis	75 Gm., nodular goiter, focal hyperplasia	18 Gm.; 0.7-cm. cortical adenoma	surgically absent 20 years		chronic cystic mastitis	14 millicuries I^{131} in 3 mos.; insulin for yrs.	tr - tr	+ - +	+ - 0
15	64	0/0	coronary thrombosis, saddle embolus	30 Gm., low epithelium	thin cortex, lipoid depletion	stromal hyperplasia	cystic hyperplasia	normal		++ - ++	0 - 0	0 - 0
16	65	1/?	diabetes, cerebrovascular accident	14 Gm., active	14 Gm., coarse lipoid	atrophic	atrophic	normal	insulin for yrs.	+ - +	++ - ++	0 - 0
17	78	0/0	ca. of breast, cerebrovascular accident	8 Gm., cuboidal epithelium	17 Gm., coarse lipoid	atrophic, cysts	no data	ca.	estinyl estradiol, 5 mg./day for 18 mos.; stilbestrol, 15 mg./day for 4 mos.; testosterone, 300 mg./wk. for 2 mos.; no therapy for 5 wks.	0 - 0	0 - +	0 - 0
18	79	?	ca. of bladder	19 Gm., high epithelium	16 Gm.; 1.5-cm. adenoma	moderate amt. stroma	polyps	chronic cystic mastitis		+ - +	+ - +	0 - 0

* Para/gravida.

† Stored gonadotropins in hypophysis assayed in male (M) and female (F) rats.

TABLE 3. STAINING REACTIONS OF CHROMOPHIL GRANULES OF ADENOHYPOPHYSIS (FORMALIN-$HgCl_2$ FIXATION)

Technique	Normal basophils	Amphophils	Acidophils
PAS—Orange G	Deep red	Rose, with rare pale orange granules	Orange
Modified Mallory aniline blue	Blue	Pale blue, with occasional red granules	Red
Chromotrope-aniline blue	Blue	Mixed red and blue	Red
Hematoxylin & eosin	Purple	Pale violet	Pink
Acid hematein	Colorless, with few fine black granules (mitochondria)	Shades of gray	Jet black

basophils, although to a less intense degree, By manipulation of pH and differentiation of a trichrome stain, the granules can be made to appear either blue or red. They can be stained with acid hematein like normal acidophils, although the reaction is usually much weaker. Occasional amphophils may be encountered which contain both Schiff positive and simple protein granules.

4. *Hypertrophic amphophils* (11)): These cells are characterized by giant nuclei which are frequently bilobed or of irregular shape and may contain a prominent nucleolus (Fig. 2). They are derived from the sparsely granulated Schiff positive cells through enlargement of the nucleus and total degranulation of the cytoplasm.

5. *Acidophils*: These are rounded, densely granular cells which are most abundant in the posterolateral portions of the anterior lobe. The simple protein granules are Schiff negative and stain with fuchsin, Orange G, and acid hematein (Fig. 1).

6. *Chromophobes*: These are apparently agranular cells which are usually much smaller than the cell types previously described. They are differentiated from the agranular hypertrophic amphophils by the size of the nucleus.

The staining reactions of the chromophil granules to the battery of techniques used in this study are summarized in Table 3, which brings out the relation of the "amphophils" to both "basophils" and "acidophils."

RESULTS

The results of the bioassays are summarized in Table 4. The differential cell counts on these 18 patients are shown in Table 5, arranged in groups according to the degree of gonadotropic activity.

Gonadotropins

The adenohypophyses of 6 patients (Nos. 1, 4, 9, 10, 13 and 15) were found to contain large quantities of gonadotropins, sufficient to cause luteinization of the ovaries and approximately a 300 per cent increase in the weight of the ventral prostate in the assay animals used. The hypophyses of 6 patients (Nos. 5, 6, 7, 11, 16 and 18) in comparable test amounts con-

TABLE 4. BIOASSAYS OF HUMAN ADENOHYPOPHYSES. AVERAGE ORGAN WEIGHTS OF TEST ANIMALS EXPRESSED AS PERCENTAGES OF CONTROLS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
								Male Rats										
Body weight	98	92	92	95	86	95	100	97	102	95	98	97	97	102	92	95	90	98
Testes	113	152	120	209	110	125	171	99	254	167	255	108	175	121	160	157	103	136
Seminal vesicles	192	196	162	299	120	141	149	111	263	369	214	122	292	119	261	180	115	172
Ventral prostate	273	220	154	314	137	148	246	103	357	354	275	162	364	143	318	188	110	249
Thyroid	96	102	98	87	98	102	108	104	94	96	91	98	102	108	95	148	96	118
Adrenals	98	91	98	128	102	90	98	94	98	97	90	98	93	112	103	100	88	100
								Female rats										
Body weight	95	92	93	97	88	89	95	97	100	88	95	98	98	88	92	91	97	97
Ovaries	514	192	101	316	282	257	530	106	595	263	264	134	684	214	384	565	101	527
Follicles	++	+	sm*	++	+	+	++	sm	++	++	++	sm	++	sm	++	++	sm	++
Corpora lutea	+	0	0	+	0	0	0	0	+	+	0	0	+	0	+	0	0	0
Uteri	339	206	173	302	367	270	264	169	427	215	367	228	504	189	350	264	118	347
Vagina	op**	clo†	clo	op	op	op	op	clo	op	op	op	clo	op	clo	op	op	clo	op
Thyroid	122	100	98	102	116	102	96	114	100	108	113	104	98	114	100	220	113	108
Adrenals	93	103	100	125	91	103	97	81	97	102	105	95	88	93	98	97	97	93

* = small.
** = open.
† = closed.

TABLE 5. DIFFERENTIAL CELL COUNTS ON ADENOHYPOPHYSES OF 18 PATIENTS, ARRANGED IN ORDER OF GONADOTROPIC POTENCY

Case No.	Sex	Age (yrs.)	Hours post-mortem	No. of cells counted	Wt. of hypophysis (Gm.)	Normal basophils %	Amphophils %	Acidophils %	Chromophobes %	Hypertrophic amphophils %	Hyaline basophils %	Remarks
1. Corpora lutea in female test rats; wt. of ventral prostate inc'd about 300% in male test rats												
1	M	19	15	3825	0.58	9.7	8.1	44.5	35.2	2.5	0	
4	M	66	5	1817	0.52	17.3	14.2	36.8	30.2	1.5	0	
9	M	82	5	2050	0.50	20.5	13.1	26.1	36.1	4.2	0	
10	F	30	10	5684	0.98	5.5	11.7	31.9	49.0	1.9	0	
13	F	62	8	2442	0.35	8.6	13.5	52.2	23.9	1.8	+	Bilateral oophorectomy
15	F	64	16	3051	0.55	11.4	13.9	37.6	33.4	2.5	1.2	
				Avge.	*0.58*	*12.2*	*12.4*	*38.2*	*34.6*	*2.4*	*0.2*	
				*S.D.**	*0.193*	*5.51*	*2.88*	*8.41*	*7.68*	*0.64*	—	
2. Large follicles but no corpora lutea in test females; ventral prostate stimulated in males												
5	M	71	20	2111	0.50	14.5	12.6	39.0	30.5	3.4	0	
6	M	72	3	2742	0.72	27.6	7.9	20.3	40.3	3.9	0	
7	M	73	8	2852	0.60	13.1	12.2	29.7	41.6	3.4	0	
11	F	32	2	3242	0.54	13.3	2.2	19.4	63.9	1.2	+	
16	F	65	10	3411	0.81	8.9	4.4	23.9	59.5	1.5	1.8	Diabetes; insulin
18	F	79	11	2802	0.68	10.4	17.9	38.3	31.3	2.1	0	
				Avge.	*0.64*	*14.6*	*9.5*	*28.4*	*44.5*	*2.6*	*0.3*	
				S.D.	*0.107*	*6.08*	*5.31*	*7.95*	*12.90*	*1.03*	—	
3. Vaginae of test females closed; slight to minimal stimulation of accessories in males												
2	M	50	7	3118	0.48	6.6	8.6	13.5	69.9	1.4	+	Triethylene phosphoramide
3	M	63	11	2182	0.47	20.9	10.4	20.4	46.4	1.9	+	Cortisone
8	M	80	4	2214	0.47	6.0	9.3	45.0	37.9	1.8	+	Cortisone, ACTH
12	F	54	8	2628	0.63	9.3	6.2	42.8	41.2	0.5	0	Estrogens, testosterone
14	F	63	5	2827	0.45	14.0	7.2	21.4	52.9	3.3	1.2	Diabetes; insulin, oophorectomy, I^{131}
17	F	78	11	2251	0.68	11.3	8.5	49.4	30.0	0.8	0	Estrogens, testosterone
				Avge.	*0.53*	*11.3*	*8.4*	*32.1*	*46.4*	*1.6*	*0.2*	
				S.D.	*0.090*	*5.06*	*1.36*	*14.01*	*11.77*	*0.91*	—	

* Standard deviation.

tained enough gonadotropin to cause definite follicular growth and a significant but less marked degree of stimulation of the male sex organs. The hypophyses of 6 patients (Nos. 2, 3, 8, 12, 14 and 17) caused only minimal stimulation of the gonads of the test rats, the vaginae of the females remaining closed.

There was no systematic variation in gonadotropic hormone content with sex, approximately equal numbers of males and females being found in each of the three groups. The ages of the patients with the highest gonadotropic hormone content ranged from 19 to 82 years, of those in the intermediate group from 32 to 79 years, and of those with minimal hormone content from 50 to 80 years. There was no significant difference in the average hypophyseal weight among the three groups.

Although there was considerable variation in the time elapsing after death before the hypophysis could be removed, this factor could not be correlated with gonadotropic potency. Those hypophyses with the most stored hormone were removed from five to sixteen hours after death; those with the least, from four to eleven hours after death.

As was expected, a wide range of percentages of the various cell types was found in the differential cell counts. The normal basophils varied from 6.0 to 27.6 per cent of the total cell count, the amphophils from 2.2 to 17.9 per cent, the acidophils from 13.5 to 52.2 per cent, the chromophobes from 23.9 to 63.9 per cent, and the hypertrophic amphophils from 0.5 to 4.2 per cent. Hyaline basophils were seen in 8 of the 18 hypophyses and were comparatively abundant in 3 instances.

There was considerable overlap in the range of differential counts found in the three groups of patients with different levels of gonadotropic potency, as may be seen from the size of the standard deviations from the arithmetical mean. Gonadotropic potency could not, therefore, be predicted on the basis of relative percentage of any cell type in an individual hypophysis. However, when the average values obtained in the high and low potency groups (Groups 1 and 3, Table 5) were compared by Student's T-test, the reduction in amphophils and hypertrophic amphophils in the latter was significant at the 1 per cent level. There was no significant difference in the other granulated cells. This suggests, though it by no means proves, that the amphophils and their derivatives, rather than either conventional basophils or acidophils, may be concerned in the production of gonadotropic hormones in man.

There was also a positive correlation between the bioassays and hypophyseal cytology. Those adenohypophyses with sufficient gonadotropic hormone content to cause luteinization in the test animals at the dosage level used had large vesicular nuclei in the amphophils and chromophobes. Nucleoli tended to be prominent. These cells resembled those characteristically associated with active protein synthesis (12). In those hypophyses in which only traces of gonadotropins were found, the nuclei were smaller and the cytoplasmic granules of the amphophils often had a pale, smudged appearance. The normal basophils remained intensely Schiff positive. There was marked pyknosis in the acidophils and often in the chromophobes (compare Figs. 3 and 4).

Perhaps the most interesting observation had to do with those glands in which very low levels of gonadotropic hormone were found. All 6 of these patients had been subjected to some sort of endocrine or chemotherapy. Two of the men (Nos. 3 and 8) had received cortisone or ACTH. Two of the women (Nos. 12 and 17) had been treated with estrogens and testosterone for carcinoma of the breast, and the third (No. 14, a diabetic) had

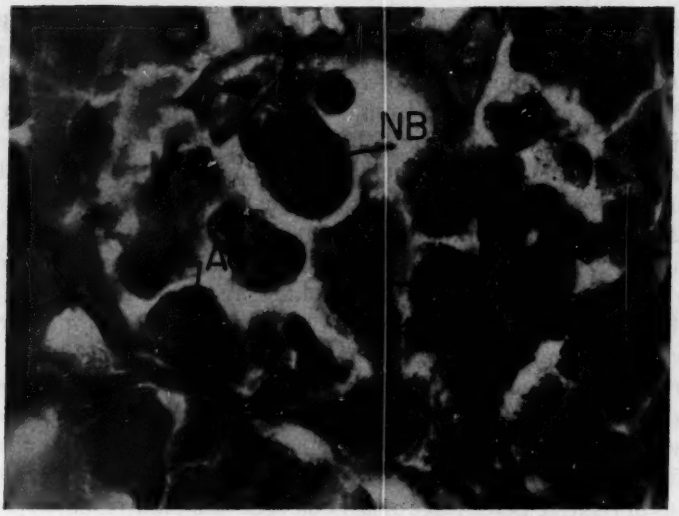

FIG. 3. Hypophysis of Patient 12, a 54-year-old female with carcinoma of the breast, who was treated with testosterone and estinyl. Note small pyknotic nuclei in acidophils (**Ac**) and persistence of Schiff positive basophils (**NB**). Minimal gonadotropins; no TSH or ACTH. (Periodic-acid Schiff-Orange G, ×750.)

received 14 millicuries of radioactive iodine for thyrotoxicosis three months before death. The sixth patient in this group was a 50-year-old man (No. 2) who had received triethylene phosphoramide (TEP) shortly before death in an attempt to arrest the growth of a malignant melanoma. TEP is a cytotoxic agent similar to those known to cause testicular damage in laboratory animals (13). Although there was not enough gonadotropin present in this hypophysis to cause vaginal opening in the female assay animals, the level of gonadotropin was considerably higher than in the other 5 patients in the group, as shown by some follicular growth in the females and a moderate degree of stimulation of the male sex organs.

In spite of the low levels of gonadotropins, all 6 of these hypophyses contained abundant Schiff positive cells and acidophils. This correlates with previous work on the hypophysis of women receiving heavy estrogen therapy for carcinoma of the breast, in whom abundant chromophils were consistently found, although other endocrine glands suggested hypophyseal suppression (14).

There was little correlation between stored gonadotropin and the state of the gonads at the time of autopsy. Two of the men with high gonadotropins had active spermatogenesis (Nos. 1 and 4); one had little or none (No. 9). High gonadotropins were found in a young woman with a large corpus luteum and a normal menstrual cycle (No. 10), in a postmenopausal

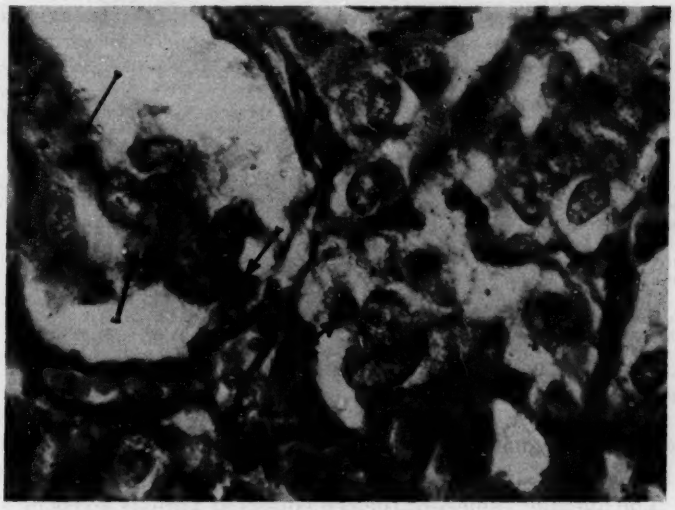

FIG. 4. Hypophysis of Patient 9, an 82-year-old male with coronary thrombosis following prostatectomy, and severe chronic thyroiditis. Note prevalence of very large vesicular nuclei. Arrows point to Schiff positive globules in the amphophils. Very high gonadotropins; no TSH or ACTH. (Periodic-acid Schiff-Orange G, ×750.)

woman with intense stromal hyperplasia (No. 15), and in an elderly castrated female (No. 13). The other castrated female studied (No. 14) had almost no stored gonadotropins—what effect the radioactive iodine she had received may have had on this condition cannot be determined from our data. There was no systematic relationship between the occurrence of gonadotropins and thyrotropic hormone or ACTH in individual hypophyses.

Thyrotropin (TSH)

The hypophysis of 1 patient (No. 16) contained enough thyrotropic hormone so that the thyroids of the male and female assay animals weighed 148 and 220 per cent respectively, of the control thyroids. In 2 hypophyses (Nos. 14 and 18) there was sufficient TSH so that the thyroids of both male and female test rats weighed approximately 110 per cent of the controls. These 3 glands were considered definitely positive for TSH. The hypophyses of 5 patients (Nos. 1, 5, 8, 11 and 17) contained enough tropic hormone so that the thyroids of the female test animals weighed over 110 per cent more than the thyroids of the controls, whereas the thyroids of the male rats fell below this figure. It was felt that these glands showed questionable TSH activity. No TSH was detected in the remaining 10 hypophyses studied.

TABLE 6. AVERAGE VALUES OF DIFFERENTIAL CELL COUNTS IN ADENOHYPOPHYSES OF PATIENTS WITH VARIOUS LEVELS OF THYROTROPIC HORMONE ACTIVITY

TSH activity		Wt. of hypophysis (Gm.)	Normal basophils %	Amphophils %	Acidophils %	Chromophobes %	Hypertrophic amphophils %	Hyaline basophils %
Stimulation of thyroid in male & female test rats (Pts. 14, 16, 18)	Avge.	0.65	11.1	9.8	27.9	47.9	2.3	1.0
	S.D.*	0.149	2.14	5.82	7.45	12.04	0.75	—
Stimulation of thyroid in female test rats only (Pts. 1, 5, 8, 11, 17)	Avge.	0.55	11.0	8.1	39.5	39.5	1.9	
	S.D.	0.074	2.98	3.37	10.56	12.55	0.93	
No stimulation of thyroid in any test animals (Pts. 2, 3, 4, 6, 7, 9, 10, 12, 13, 15)	Avge.	0.58	14.1	11.2	31.2	41.2	2.3	
	S.D.	0.164	7.05	2.64	11.07	11.92	1.13	

* Standard deviation.

As was true of gonadotropins, there was no correlation between thyrotropic activity and the relative percentage of any cell type in individual glands. The average values for the cell counts at various levels of TSH activity are shown in Table 6. There were no significant differences found between high and low potency glands in the grouped data. All 3 of the patients who showed definite TSH were females but, in a sample of this size, little significance can be attached to this finding. The fact that both the diabetics (Nos. 14 and 16) showed TSH is, perhaps, significant in view of the frequent association of diabetes with thyroid disease. One of these women (No. 14) had, in fact, a 75-gram multinodular goiter and had received radioactive iodine for thyrotoxicosis. Questionable thyrotropic activity was detected in 2 patients who had received some hormone therapy (Nos. 8 and 17). The thyroid of Patient 12, who had been treated with large doses of testosterone and terminally with estinyl, was, however, notably atrophic (Fig. 5).

The correlation between TSH detected in the hypophysis and the structure of the thyroid was much better than that between gonadotropins and the structure of the gonads. The thyroid of Patient 16, who had the highest level of TSH, was easily distinguished from all others by the small follicles and abundant scalloping of the colloid (Fig. 6). Patients 14 and 18, who also showed TSH, had histologically active thyroid glands, although to a less intense degree. The thyroid epithelium of Patient 14 showed the anisonucleosis which is characteristic of radioactive iodine treatment (Fig. 7). Those patients with questionable TSH showed foci of activity alternating with inactive areas, with the exception of Patient 11, in whom the epithelium was predominantly flat. Those patients with no TSH all had very low thyroid epithelium and little or no scalloping in the colloid.

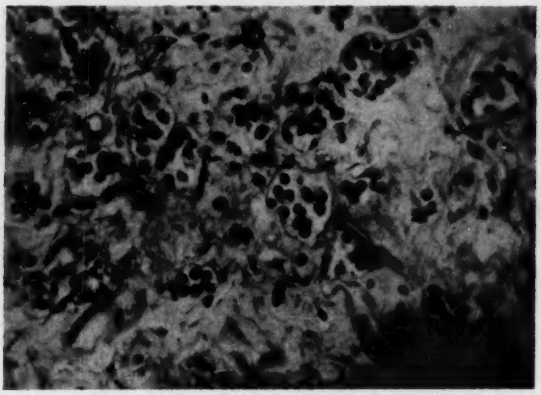

FIG. 5. Thyroid of patient 12, a 54-year-old female with carcinoma of the breast, who was treated with testosterone and estinyl. Note extensive fibrosis and tiny, atrophic follicles. No stored TSH. (Hematoxylin and eosin, ×100).

Patient 9 is of particular interest. Although this 82-year-old man had no clinical history suggesting an endocrine disorder, at autopsy his thyroid was almost completely fibrosed, only rare degenerating follicles surrounded by lymphocytes remaining (Fig. 8). The high levels of Schiff positive cells and of hypertrophic amphophils in his hypophysis suggest a compensatory reaction to end-organ failure, but the only tropic hormone found at assay was a very high level of gonadotropin.

No single histologic feature could be correlated with TSH activity in

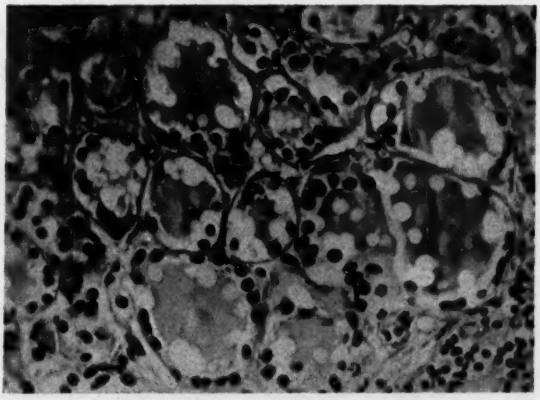

FIG. 6. Thyroid of Patient 16, a 65-year-old female diabetic who had a cerebrovascular accident. Note small follicles and scalloped colloid. High titer of TSH in hypophysis. (Hematoxylin and eosin, ×100.)

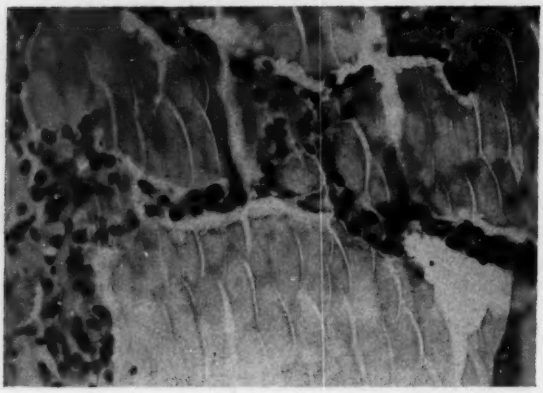

FIG. 7. Thyroid of Patient 14, a 63-year-old female with diabetes, mesenteric thrombosis, and thyrotoxicosis treated by I^{131}. Note variable nuclear size. TSH in hypophysis. (Hematoxylin and eosin, ×100.)

these patients. Although all the hypophyses with positive TSH contained cells with large Schiff positive globules, similar cells were also abundant in Patient 9 and were occasionally seen in other patients in whom TSH was not found (Fig. 4).

Adrenocorticotropic hormone (ACTH)

Definite evidence of ACTH storage was found in 1 patient (No. 4); it may also have been present in Patient 14. The differential cell counts in these cases were in no way distinctive. Patient 4 had 25-gram adrenals and

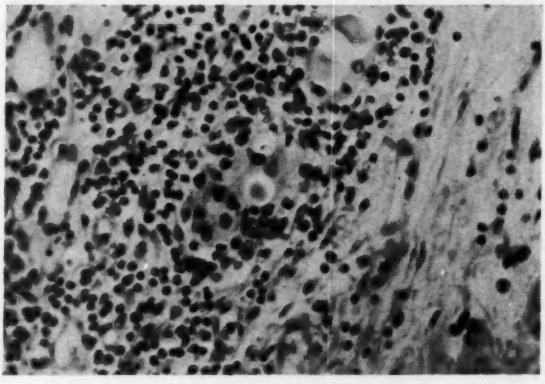

FIG. 8. Thyroid of Patient 9, an 82-year-old male with coronary thrombosis and chronic thyroiditis. Note sparse, small follicles surrounded by lymphocytes and extensive fibrosis. High gonadotropins but no TSH in hypophysis. (Hematoxylin and eosin, ×100.)

Patient 14 had 18-gram adrenals with a 0.7 cm. cortical adenoma. However, large cortical adenomas were also present in Patients 13 and 18, and Patients 3 and 8 had adrenals weighing over 20 grams. No ACTH was detected in any of these last four hypophyses.

DISCUSSION

In the work reported here it has been assumed that the two halves of the hypophysis were bilaterally symmetrical with respect to cell distribution. This, of course, may not have been true in all cases and may have been a source of error. Although the assay method used is a sensitive and reliable test for gonadotropins, as shown by the agreement between results in male and female test animals (Table 4), it is a much less sensitive indicator of TSH and ACTH activity. Although the positive assays of these substances can probably be accepted as valid, the negative assays do not exclude the presence of small amounts of these hormones.

Bioassays for any tropic hormone measure only stored material and give no information about the production and release of these substances. There is much circumstantial evidence, however, linking cells resembling our amphophils with active hormone production. Thus Mellgren reports that "sparsely granulated basophils" are often increased in human adrenal hyperactivity and that the hypertrophic amphophils which are derived from them are constantly increased (11). Large numbers of finely granulated basophils are also characteristic of Addison's disease in man (11, 15). These cells are known to be Schiff positive (8). Normal basophils and acidophils appear to decrease in Addison's disease (16, 17).

A striking hyperplasia of sparsely granulated Schiff positive cells has been reported both in human myxedema (8) and in a case of thyroid agenesis (7). A similar hyperplasia of finely granulated Schiff positive cells has been reported in rats following total thyroidectomy (5). Acidophils decrease in myxedema and in thyroidectomized animals (18).

The changes in the hypophysis following castration are somewhat more controversial. In animals it is generally accepted that gonadectomy is followed by an increase in cells with a morphologic resemblance to our "normal basophils," which are commonly accepted as the source of FSH (5, 18). In some laboratory animals these basophils eventually form vacuolated signet-ring castration cells, which are not seen in man. It is generally conceded that the gonadotropic potency of the animal hypophysis increases after castration.

In man, Biggart has reported that normal basophils and large pale cells containing sparse basophil granules increase following castration (19). The older German literature, however, reports either an "unripe acidophil" or a "hypertrophic chromophobe" as characteristic of human castration

(20, 21), testicular deficiency (21, 22), or ovarian agenesis (23, 24). In view of the morphology and amphophilic staining properties of the sparsely granulated Schiff positive cells, it seems probable to us that this cell type, rather than the well granulated acidophil or the agranular chromophobe, is the one involved. True acidophils seem to decrease following castration (18).

The gonadotropic assays in this study have not been broken down into assays for follicle-stimulating hormone (FSH) and for luteinizing hormone (LH), by design. Although there seems to be good biologic evidence for the existence of two gonadotropic hormones, their chemical separation is extremely difficult, especially since neither FSH nor LH has ever been produced in indisputably pure form. Our hypophyses with the highest titers of gonadotropic activity (Group 1, Table 5) would be generally conceded to contain both FSH and LH, and the intermediate group certainly shows FSH activity and a lesser degree of LH. It is difficult to relate this to any quantitative or qualitative difference between the two groups.

Another point which must be considered in evaluating these results is the use of the Schiff reaction. Although FSH, LH and TSH all have been shown to contain glycoprotein which should give a positive Schiff reaction, the test itself is far from specific and will give a positive result with a wide variety of complex substances (25). Our results indicate that not all Schiff positive material in the hypophysis represents active tropic hormone. The Schiff positive "normal basophils" and "amphophils" found in inactive hypophyses may well represent biologically inactive intermediate or storage forms of hormone.

This work gives no clue as to the function of the Schiff negative acidophils which were present in large numbers in both very active and completely inactive hypophyses. No final conclusions concerning the morphologic evidence for the presence of ACTH and TSH seem justified on the basis of this material.

SUMMARY

The adenohypophysis was removed at autopsy from 9 men and 9 women who had died of a wide variety of diseases. One sagittal half of each hypophysis was assayed in hypophysectomized male and female rats for the presence of gonadotropins, thyrotropic hormone and adrenocorticotropic hormone. A differential cell count was performed on the other half, using sections stained by the periodic-acid Schiff reaction and Orange G.

The hypophyses of 6 patients caused ovarian luteinization with the test dose used, 6 others contained enough gonadotropin to produce significant follicular growth without luteinization, and the remaining 6 contained little or no gonadotropin. All patients in the last category had received some

type of hormone or chemotherapy. Gonadotropic potency could not be predicted from the relative percentage of any cell type in an individual hypophysis, but the 6 patients with the highest levels of stored gonadotropins had significantly higher average values of sparsely granulated Schiff positive amphophils and of hypertrophic amphophils than did the 6 patients with little or no stored gonadotropin. There was no significant difference in average values of normal basophils or acidophils. This suggests that the amphophils may be the source of some gonadotropic hormone in man.

Undoubted thyrotropic activity was demonstrated in 3 hypophyses. Adrenocorticotropic activity was definitely found in one hypophysis and was questionably present in another. However, no specific cytologic features could be correlated with the presence of either TSH or ACTH.

Gonadotropins, TSH and ACTH were noted to occur independently of one another. There was no obvious correlation between the level of any tropic hormone and the age or sex of the patient or the hypophyseal weight.

Although it was concluded that a positive Schiff reaction *per se* cannot be accepted as histochemical proof of the presence of biologically active hormone, the evidence linking the sparsely granulated Schiff positive cells to tropic hormone production has been reviewed.

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AN EXPERIMENTAL MODEL ILLUSTRATING THE PATHOGENESIS OF THE DISEASES OF ADAPTATION

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WHEN the pituitary-adrenocortical axis is activated during the general adaptation syndrome, various types of inflammatory responses are suppressed. This had been demonstrated first for the so-called histamine appendicitis (1, 2), several varieties of acute inflammatory lung edema (3, 4) and the anaphylactoid inflammation caused by egg-white in the rat (5, 6). It undoubtedly holds true also for many other experimental and clinical instances of inflammation. However, subsequently it was noted that—at least under certain "conditioning" circumstances—systemic stress can also facilitate the production of inflammatory lesions (*e.g.*, nephritis and myocarditis after exposure to cold). The adrenals were thought to play an important part in both these responses, since adrenalectomy prevented the inhibition, as well as the stimulation of inflammation by systemic stress (7).

Later, when pure adrenal corticoids became available, it was observed that some, the so-called mineralo-corticoids (*e.g.*, desoxycorticosterone) stimulate, whereas others, the so-called gluco-corticoids (*e.g.*, cortisone or hydrocortisone) inhibit inflammation (8).

Thus gradually, the concept began to take shape that a major factor in the pathogenesis of many diseases is an inappropriate adaptive response to certain pathogens, a "derailment of the general adaptation syndrome." Of course stress plays a part in the pathogenesis of most (if not all) maladies, but the inflammatory (*e.g.*, rheumatic) diseases are, perhaps, the clearest examples of such "diseases of adaptation," because their course is most readily influenced by stress itelf and by treatment with those hormones which are produced in excess during stress (*e.g.*, ACTH, hydrocortisone).

The many investigations and discussions instigated by this concept have been surveyed at length in two monographs (8, 9) as well as in a series of "Annual Reports on Stress" (10, 11, 12). In connection with the experiments to be described here, let us merely call special attention to one recent experimental study, which demonstrated that the suppression, by

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hydrocortisone, of inflammatory responses to local irritants is not necessarily beneficial. Indeed it was shown that the very absence of inflammation in response to local stress facilitates the production of a much more serious type of tissue damage, namely, necrosis (13).

Nevertheless, several serious objections have been raised against the whole concept of the diseases of adaptation. Perhaps the most important among these are the following:

1. It is difficult to understand how the same factor, systemic stress, could both inhibit and produce disease manifestations (14).
2. Even if injected corticoids combat disease manifestations, it remains to be shown whether endogenous adrenal hormones, secreted in response to stress, could be effective in this sense (15, 16).
3. Even if injected corticoids can cause disease, it remains to be shown whether endogenous adrenal hormones, secreted in response to stress, could be pathogenic at the dose level and under the circumstances under which they are produced (15, 16).
4. It has been shown that temporary treatment with a mineralo-corticoid (desoxycorticosterone) can cause delayed, so-called "meta-corticoid" lesions (*e.g.*, nephrosclerosis, hypertension) in experimental animals. This implies that, even in diseases due to an excess of such hormones, the corticoid concentration in the blood and urine may have returned to normal by the time the pathologic changes are fully developed. Yet, it remains to be seen whether natural adrenal hormones, secreted in response to stress, could ever exert delayed pathogenic actions of this kind (17).
5. Finally it is not quite clear just how the systemic (*e.g.*, shock) and topical (*e.g.*, inflammation, necrosis) manifestations of stress are interrelated.

In view of these uncertainties it appeared of interest to devise an experimental model, in which all these points could be tested by objective indicators. To permit generalizations, some simple pathogen should be used—one which produces fundamental disease manifestations common also to conditions encountered in clinical medicine. The basic elements of such a model would have to be: the "pathogen" or topical stressor (the principal immediate cause of disease), the direct effect of the topical stressor (the local disease manifestations), the systemic stressor (which might influence the disease manifestations) and the adrenals (through which the actions of the systemic stressor might be mediated).

The object of this communication is to report upon the outcome of such an experiment.

MATERIALS AND TECHNIQUES

Experimental animals.—Forty-eight female albino Wistar rats, weighing 149–190 Gm. (average 158 Gm.) were subdivided into four groups as follows:

I: intact controls,
II: intact animals exposed to systemic stress,
III: adrenalectomized controls,
IV: adrenalectomized animals exposed to systemic stress.

The topical stressor.—In all four groups a "granuloma pouch" was produced by the usual procedure, as described elsewhere (13). In essence, it consists in the injection of 25 ml. of air, under the shaved dorsal skin, this being immediately followed by the injection of 0.5 ml. of 0.25 per cent croton oil solution (in corn oil).

Normally, this intervention leads to the transformation of the connective tissue, which lines the air-space, into a thick granuloma membrane. Then, the air is gradually displaced by a hemorrhagic inflammatory exudate, within the next eight to ten days.

This dilute croton oil solution acted as a pathogen, causing a predominantly inflammatory topical response, similar in its essence to that seen in various spontaneous diseases (*e.g.*, after infections, chemical irritants, allergens or traumas).

On the eighth day of the experiment, a second dose, this time of 0.5 ml. of a 50 per cent croton oil solution (in corn oil) was introduced into the cavity of the granuloma pouch, again in all the animals of the four groups. This strong solution of the irritant normally produces tissue necrosis, an effect likewise commonly observed in the topical manifestations of various spontaneous diseases in man.

Assessment of the direct effects of the topical stressor.—The degree of *inflammation* produced by the first injection of dilute croton oil was assessed on the basis of the amount of exudate formed. This can readily be appraised throughout the duration of such an experiment, even in the living animal, by merely transilluminating the granuloma pouch with an electric flashlight. In a darkened room, the upper level of the red hemorrhagic exudate is clearly distinguishable from the transparent air-remnant and its height can be traced on the skin with an eyebrow pencil. This facilitates the objective assessment of the rate of exudate formation, by comparing the amount day-by-day.

Necrosis is even more readily appraised by simple inspection because the dead-skin regions take on a characteristic grayish-brown color, quite different from the pink shade of the surrounding healthy skin.

The systemic stressor.—As a systemic stress-producing agent, we decided to use forced restraint of free motion, because it does not necessitate the introduction into the body of any foreign materials or the infliction of traumatic tissue injuries. The technique used consisted in restraining the movements of the rat, by fastening its four limbs to a wooden board, with adhesive tape, the animal lying flat on its abdomen. The ends of the upper incisors were clipped, just before immobilization, to prevent the animals from injuring themselves while trying to chew on their bandages.

This procedure is irritating and provokes a certain amount of struggling. Thus it causes systemic stress, predominantly through neuromuscular exhaustion. The condition of the animal is rather comparable to that occasioned by extreme physical and mental fatigue in man.

Stress was thus induced in the animals of Groups II and IV, by forced restraint of free motion between the seventy-second and the ninetieth hour after the production of the granuloma pouch. The fourth day was selected, because previous experiments had shown that it is during this "critical period" that gluco-corticoids can most effectively inhibit inflammatory phenomena in the granuloma pouch (18).

Adrenalectomy.—Bilateral adrenalectomy was performed through the dorsal approach in all the animals of Groups III and IV, on the first day of the experiment, six hours prior to the production of the granuloma pouch.

Since adrenalectomized rats are notoriously irresistant to stress, the animals of Group IV had to receive some type of substitution therapy. It was not thought advisable, how-

ever, to give them any treatment which the other rats would not receive. Hence, we gave 1 per cent NaCl as a drinking fluid and 1 mg. of desoxycorticosterone acetate microcrystals (in 0.1 ml. of vehicle daily, subcutaneously) throughout the experiment, to the animals of all four groups.[1]

Thus the animals of Groups III and IV were not wholly deprived of corticoids, but they were prevented from producing any endogenous adrenal hormones in response to stress.

RESULTS

In Figure 1, the arrangement of the experiments is summarized and the principal results are illustrated by the photographs of 4 rats, each representing the average appearance of its respective group, on the eighth day (after treatment with dilute croton oil) and on the tenth day (after treatment with strong croton oil).

On the eighth day after exposure to the initial, mild irritation with 0.25 per cent croton oil, the intact controls (Group I) exhibited a well developed, ellipsoid granuloma pouch readily visible through the skin. Transillumination of this structure, with the rat held in a vertical position, showed the fluid-level to be at the height indicated by the eyebrow-pencil mark. In other words, the "pathogen" (dilute croton oil) produced a measurable disease manifestation (exudative inflammation).

In the intact stressed animals (Group II), this disease manifestation was inhibited, in that no exudate could be found in any of the rats of this group by transillumination. It will also be noted that no ellipsoid granuloma-pouch structure formed underneath the skin. The slight prominence of the corresponding region is merely due to residual air.

In all the adrenalectomized controls (Group III) and adrenalectomized stressed (Group IV) animals, as in the 2 rats shown here, the granuloma pouch was slightly larger than in the intact controls and contained somewhat more exudate (pencil mark). Here, however, there was no difference between the stressed and the nonstressed animals.

In order to obtain an accurate quantitative evaluation of exudation at this point of the experiment, it would have been necessary to remove the exudate and measure its volume. We did not wish to do this, since it would have interfered with the second half of the investigation, to be described. However, it may be said, on the basis of the transillumination test, that none of the animals in Group II showed any visible exudate, whereas exudate was present in all the animals of all other groups.

Evidently, under these conditions, the beneficial antiphlogistic effect of systemic stress was dependent upon the presence of the adrenal glands.

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[1] Preliminary experiments had shown us that such small amounts of desoxycorticosterone with a moderate excess of NaCl intake did not noticeably change tissue reactivity to croton oil, but did suffice to maintain adrenalectomized rats in good condition.

Group	I	II	III	IV
First day	0.25% Croton oil	0.25% Croton oil	Adrenalectomy 0.25% Croton oil	Adrenalectomy 0.25% Croton oil
Third day	———	Stressed	———	Stressed
Eighth day				
Eighth day	50% Croton oil	50% Croton oil	50% Croton oil	50% Croton oil
Tenth Day				

FIG. 1.—An experimental model illustrating the pathogenesis of the diseases of adaptation.

Immediately after taking this picture, a strong (50 per cent) croton oil solution was introduced into all the granuloma pouches. Within forty-eight hours (by the tenth day) in the intact controls, adrenalectomized controls and adrenalectomized stressed animals (Groups I, III and IV), this strong irritant caused only an additional accumulation of exudate. Its amount is indicated by the upper, thick pencil stroke.

Only in the intact stressed animals (Group II) was the reaction to this strong pathogen of an essentially different character. We note that now, even here, some exudate began to accumulate (black line) and there was also some inflammatory induration of the connective tissue around the air space, as judged by palpation. However, almost the entire covering skin had undergone massive necrosis, and exhibited a reddish-brown discoloration in all the animals of this group. No necrosis was seen in any of the animals of the other three groups, with the exception of 1 rat in Group IV, which showed a small patch of necrosis. At this time, the animals were killed and the amount of exudate was measured after aspiration into a graduated syringe. The volume of exudate in Group I was 11.6 ± 1.3 ml.; in Group II, 4.7 ± 1.9 ml.; in Group III, 17 ± 3.7 ml.; and in Group IV, 18.3 ± 2.9 ml.

Evidently the systemic stress situation to which we had exposed the animals one week earlier, was detrimental, in that it had diminished skin resistance to the necrotizing actions of a topically applied strong pathogen. This predisposing effect of stress is again dependent upon the adrenals, since it is not manifest after suprarenalectomy (Group IV).

As an incidental finding it was observed that, in adrenalectomized animals, the granuloma pouch tends to become very adherent to the skin, whereas in intact animals loose connective tissue soon separates it from the adjacent derma. Probably due to this close contiguity, slight suffusions (not visible on the photographs) did occur frequently in the adrenalectomized rats; but even in this respect there was no difference between Groups III and IV.

DISCUSSION

The information which these experiments could give concerning the much debated points enumerated in our introduction may be summarized as follows:

1. The same factor—in this case, systemic stress caused by neuromuscular exhaustion—can both inhibit and produce disease manifestations.

2. Endogenous adrenal hormones can be beneficial at the dose level and under the circumstances under which they are secreted in response to stress. This was manifested during the first part of the experiment. Here systemic stress inhibited the formation of abnormal inflammatory tissue, but only in the presence of the adrenals.

3. Endogenous adrenal hormones can be pathogenic at the dose level and under the circumstances under which they are secreted in response to stress. This was shown by the facilitation of necrosis during the second part of our experiment. Here, in the intact animal not exposed to stress, a strong inflammatory barricade developed in response to the initial mild irritation. Hence, subsequent treatment with the strong irritant caused only further inflammation, which protected the adjacent skin from the more serious condition of tissue death (necrosis). The intact stressed animal, on the other hand—precisely because an inflammatory granuloma barricade failed to develop in response to the initial mild stimulation—was unprepared to resist the subsequent, more serious local irritant (50 per cent croton oil) and showed extensive skin necrosis. This detrimental effect of systemic stress is again dependent upon the adrenals, since it does not occur after adrenalectomy.[2]

4. The activation of the adrenal glands by systemic stress can exert delayed pathogenic actions, which become manifest long after the systemic stressor has ceased to act.

5. Finally, these observations help in understanding the mechanism through which the biologic reactions of systemic and of local stress are integrated. The so-called "local adaptation syndrome "(L-A-S), the characteristic triphasic adaptive response to topical injury, involves both necrosis and inflammation. It has been described and discussed at some length by several participants of the recently held International Symposium on the Mechanism of Inflammation (19). The experiments reported in this paper furnish additional evidence in favor of the view expressed at that meeting, that a close relationship exists between the general adaptation syndrome as a systemic manifestation of stress, and the local adaptation syndrome as its topical equivalent. In particular, local stress can cause systemic stress and the latter can, in turn, greatly modify the course of the former. Indeed, various combinations of these two responses represent integral parts of many among the common maladies of man.

The question might still be raised whether all systemic stressors would

[2] These experiments definitely prove that both the beneficial (antiphlogistic) and the detrimental (pronecrotic) effects of systemic stress depend upon an endogenously produced excess of adrenal hormones. It should be emphasized, however, that they do not show to what extent this excess is due to increased production of hormones (absolute hypercorticoidism), increased conditioning to a given amount of adrenal hormone (relative hypercorticoidism), or both. In more recent experiments, adrenalectomized rats maintained with hydrocortisone (instead of desoxycorticosterone) were otherwise treated in the same manner as those described in this communication. Here, the importance of sensitization to an antiphlogistic corticoid by stress was quite evident. Doses of hydrocortisone, which failed to inhibit inflammation in the nonstressed adrenalectomized animals, became highly effective in this respect after exposure of the animals to stress.

act in this manner upon the manifestations of local stress, or whether the struggling in response to the forced restraint acts specifically. This has been the subject of subsequent investigations, which will be published[3] elsewhere; they revealed that the effects of starvation (20) and of experimental jaundice produced by complete severance of the common bile duct (21) rather closely duplicate the aforementioned actions of forced restraint.

Our experimental model is directly concerned only with the part played by the adrenals in determining the production of inflammation and tissue necrosis by a topical irritant during systemic stress. However, inflammation necrosis, topical irritants and systemic stress are fundamental elements of most, if not all, maladies. Actually, there is no evidence to prove that any tissue damage (no matter where, and no matter by what agent it be produced) would fail to be similarly regulated through the adrenals during the systemic stress of disease.

SUMMARY

Using the granuloma-pouch technique in the rat, it has been shown that, depending upon circumstances, systemic stress can either inhibit or aggravate the topical damage caused by exposure of a limited tissue area to a pathogen (*e.g.*, a chemical irritant, such as croton oil).

Both these effects of systemic stress depend upon endogenously produced adrenal hormones, since they are abolished by complete adrenalectomy.

Both these effects may be delayed and may become manifest only long after the systemic stressor has ceased to act.

The observations are discussed with a view to clarifying the manner in which the systemic and topical manifestations of disease are interrelated.

Acknowledgment

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EFFECT OF ADRENOCORTICOTROPIC HORMONE AND CORTISONE ACETATE ON THE URINARY AND BLOOD LEVELS OF ASCORBIC ACID IN MAN*

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INTRODUCTION

IT IS the purpose of this communication to elaborate on a previous preliminary (1) report and present some further observations concerning the effects of ACTH and cortisone on the metabolism of ascorbic acid.

The advent of corticotropin and the adrenocortical hormones has led to a renewed interest in the possible relationship between ascorbic acid and adrenocortical function. The many observations have recently been excellently reviewed by Pirani (2) and Bicknell and Prescott (3).

Our interest in this subject arose from the observations of Andreae, Browne and their associates (4, 5) in this Clinic, who demonstrated that in previously healthy traumatized individuals there was a fall in the blood and urinary ascorbic acid levels accompanied by a marked rise in the urinary corticoids. In conjunction with investigations into the general metabolic effects of ACTH and cortisone, it was felt desirable to observe the changes in ascorbic acid metabolism associated with the administration of these hormones.

MATERIAL AND METHOD OF STUDY

The studies to be reported were carried out on patients admitted to the metabolic ward of the Royal Victoria Hospital. The wide spectrum of disease processes studied is attributable to the concomitant investigation of the clinical effects of these hormones. The chronic disease states in which these investigations were carried out are listed in Table 1.

The patients were started on a known protein, carbohydrate, fat and electrolyte intake, as well as on one of three levels of ascorbic acid[1] intake. The dietary levels were determined from the tables in the Canned Food Reference Manual (6) and an added weighed supplement of ascorbic acid was administered orally with the patient's meals.

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* This work was supported by a grant from the National Research Council (Canada).

[1] The authors are indebted to Hoffman-LaRoche Ltd., through the courtesy of Dr. Ruth Wolfe, for the supply of ascorbic acid used in this study.

TABLE 1. NUMBER OF SUBJECTS AND DISEASE PROCESSES INVESTIGATED

Types of disease	No. of cases
1. Rheumatoid arthritis	6
2. Rheumatoid arthritis and psoriasis	1
3. Rheumatoid arthritis and bronchial asthma	1
4. Bronchial asthma	8
5. Rheumatoid arthritis and multiple myeloma	1
6. Chronic berylliosis	2
7. Silicosis	1
8. Hodgkin's lymphoma and amyloidosis	1
9. Periarteritis nodosa	1
10. Malignant exophthalmos	1
11. Ulcerative colitis	1
12. Lupus erythematosus	2
13. Pernicious anemia	1
14. Sympathetic ophthalmia	1
15. Atopic dermatitis, keratoconus	1
16. Addison's disease	1
17. Scurvy	2
Total	*32*

Thus in 19 patients the 24-hour intake of ascorbic acid was approximately 250 mg., in 2 it was approximately 1,000 mg. and in 10 no supplement was given. In the latter cases the ascorbic acid content of the diet was kept as low as was feasible, taking into consideration the other metabolic studies which were being carried out. The amount which a particular patient was to receive was in part based upon preliminary determinations of blood and urinary ascorbic-acid levels. After a preliminary control period varying from five to nineteen days, the patient was given either ACTH[2] or cortisone acetate.[3] The 24-hour dosage of ACTH varied from 20 to 200 mg., divided into four doses given at 6-hour intervals; that of cortisone acetate varied from 100 to 500 mg. per twenty-four hours, using several intramuscular injection sites at the same time. In later studies cortisone was given orally in four equally divided doses. In all instances, when possible, the study was continued after the cessation of ACTH or cortisone therapy. Two infants with clinical and x-ray evidence of scurvy were given a vitamin-C free diet; 1 received ACTH alone and the other received both ACTH and cortisone. The blood and urinary ascorbic-acid and formaldehydogenic corticoid levels were estimated during these studies.

Twenty-four-hour collections of urine were made, using as a preservative 5 cc. of glacial acetic acid. The following chemical determinations were carried out on aliquots of these urines:

1. Creatinine, by a modification of Folin's method (7). These values served as an indication of the completeness of the urine collections.

[2] This work was supported by generous allotments of ACTH from the Armour Company.

[3] We wish to acknowledge the generous supply of cortisone from "The Special Committee on ACTH and Cortisone," Ottawa.

2. Ascorbic acid was determined by the technique of Roe and Kuether (8), which is based on the estimation of the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid.

Blood ascorbic acid levels were determined at suitable intervals, and in several patients four-hour determinations were made during the first twenty-four hours of ACTH administration. The blood was collected in oxalated Pyrex glass tubes and kept in a refrigerator at a temperature of 0–1° C. until the analysis could be made. Kassan and Roe (9) have pointed out that the ascorbic acid content of plasma in contact with its red cells remains unaltered for fifty-two hours at ice box temperature. In an attempt to determine whether the alteration in ascorbic acid excretion occurring after ACTH is confined to a particular period, urine collections on the last control day and the first ACTH day were fractionated into eight-hour periods in 6 subjects.

Total blood eosinophil counts were made, using the phloxine propylene-glycol diluting fluid described by Randolph (10) and a Levy counting chamber.

RESULTS[4]

The effect of ACTH and cortisone acetate on ascorbic acid metabolism was studied in 32 subjects. The results are summarized in Table 2, and are grouped according to the ascorbic acid intake under the headings of: intramuscular ACTH, intramuscular cortisone and oral cortisone.

Since space does not permit the inclusion of all the data, the case histories of 10 representative patients, together with charts showing the ascorbic acid excretion and other pertinent data are presented (Figs. 1–10).

Random blood ascorbic acid levels were determined in selected patients and the data indicate that these levels increase when the urinary ascorbic acid excretion is increased, and that they remain low or constant when the urinary ascorbic acid excretion is low or unchanged.

In 5 patients blood ascorbic acid levels were determined at four-hour intervals on the first day of ACTH administration. The data suggest that the levels are highest during the daytime, when the whole intake of ascorbic acid occurs.

In 6 patients the urine on the day prior to, and on the first day of ACTH and cortisone administration, was fractionated into eight-hour collections. The same trend as was noted for the blood determinations is evident. The urinary ascorbic acid excretion is higher during the day than during the night. This rise during the day coincides with the total intake of ascorbic acid which is administered through the 8 a.m.–8 p.m. period.

Two opportunities to study the hormonal influence on ascorbic acid metabolism in infantile scurvy arose. The first was a 9-month-old infant with clinical and x-ray evidence of advanced scurvy, who was given a vitamin-C

[4] These are published in an M.Sc. thesis entitled "Some Metabolic Effects of Adrenocorticotrophic Hormone and Cortisone Acetate" by John C. Beck, McGill University, Montreal, Canada.

TABLE 2

Ascorbic acid intake	Response*	Questionable response	No response
27 patients receiving ACTH i.m.			
Dietary—no supplement	8	1	2
250 mg.	12	2	1
1,000 mg.	1	—	—
	—	—	—
Total	*21*	*3*	*3*
9 patients receiving cortisone i.m.			
Dietary—no supplement	1	—	3
250 mg.	2	—	2
1,000 mg.	—	—	1
	—	—	—
Total	*3*	*0*	*6*
3 patients receiving cortisone orally			
Dietary—no supplement	3	—	—
250 mg.	—	—	—
1,000 mg.	—	—	—
	—	—	—
Total	*3*	*0*	*0*

* Response = an increase in urinary ascorbic acid excretion upon the administration of ACTH or cortisone.

free diet and received 30 mg. of ACTH over a 24-hour period. The urinary ascorbic acid excretion which was less than 0.5 mg. per twenty-four hours during the control period increased to 2.3 mg. per twenty-four hours coincident with the administration of ACTH. This questionable rise was accompanied by a definite rise in the chemically determined corticoids. The blood ascorbic acid level remained at zero during the period of study. Following this short course of ACTH injections there occurred definite clinical improvement. This was accompanied by calcification in the femoral subperiosteal hematoma.

The second case was a 16-month-old male who, because of infantile eczema, had ingested only milk and sugar without supplementary vitamins and who had clinical and x-ray evidence of scurvy. There was no ascorbic acid detectable in the blood or urine during a six-day control period. Upon administration of 80 mg. of ACTH daily, ascorbic acid appeared both in the urine and in the blood. The urinary ascorbic acid reached a peak of 2.77 mg. per twenty-four hours on the first day after the cessation of ACTH in-

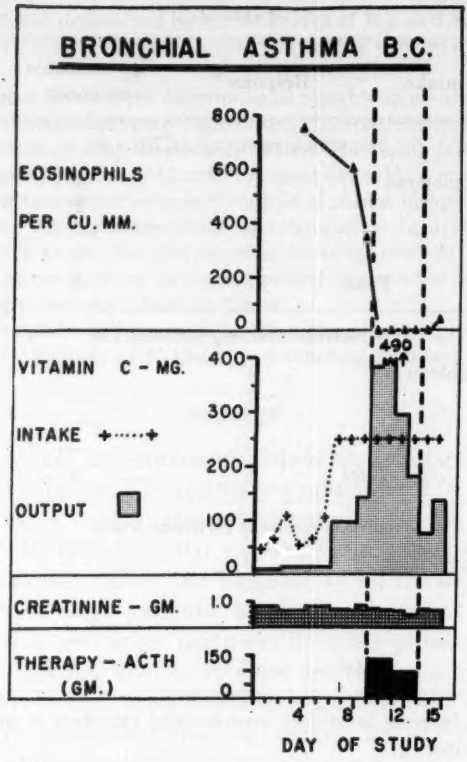

FIG. 1. *Case 8.* This patient with bronchial asthma showed a low initial excretion of 20 mg. of ascorbic acid per twenty-four hours. On increasing the 24-hour intake from an average of 75 mg. to 250 mg. on day 7, there was a prompt increase in the urinary ascorbic acid excretion to 143 mg. on day 9. On administration of 150 mg. of ACTH daily there was a further marked increase in the urinary ascorbic acid excretion, which reached a peak of 490 mg. per twenty-four hours on the second day of ACTH injection. During the four days of ACTH administration this patient excreted approximately 800 mg. of ascorbic acid above the average control level. A decrease of ascorbic acid excretion occurred upon cessation of ACTH.

jection and persisted for three days thereafter. The same phenomenon was observed during a second course of ACTH injections and ascorbic acid again appeared in the urine during a subsequent course of 75 mg. of oral cortisone daily. The largest amount excreted during this period was 1.27 mg. per twenty-four hours. The urinary formaldehydogenic corticoids also increased during the administration of ACTH. There was marked clinical improvement in the infant during the administration of either ACTH or cortisone but no alteration in the x-ray evidence of scurvy.

DISCUSSION

The limitations of balance studies were fully appreciated in planning this investigation. The estimation of the ascorbic acid content of the diet from the food tables fails to take into consideration the various sources of the diet, the length of time between harvesting and processing, and the type of preparation which the diet receives. Furthermore no information concerning the fate of ascorbic acid between ingestion and excretion is obtained.

The data nevertheless give some indication of the duration of time required for the development of a relatively constant ascorbic acid output on a known intake, a phenomenon which has been termed saturation. The

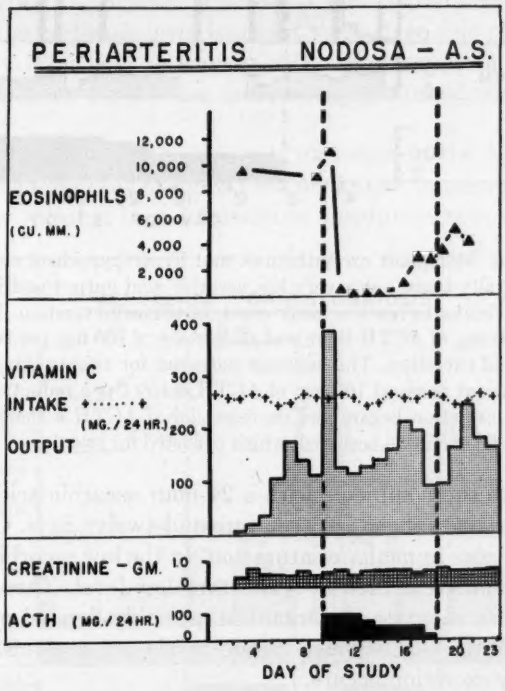

FIG. 2. *Case 12.* This patient with periarteritis nodosa was initially given ACTH. She returned to hospital five months later and received cortisone. (This second study is reported as Case 27—Fig. 8.) Initially the ascorbic acid excretion was below 20 mg. per twenty-four hours, and it required six days on a constant intake before a relatively constant urinary ascorbic acid excretion was observed. On administration of 100 mg. of ACTH daily, there was an increase of about 270 mg. in the excretion of ascorbic acid for the first twenty-four hours, followed by a return to the control level. Subsequently a slow increase in the excretion occurred until the 24-hour ACTH dosage was reduced from 50 to 25 mg., when a sharp fall in the ascorbic acid was noted.

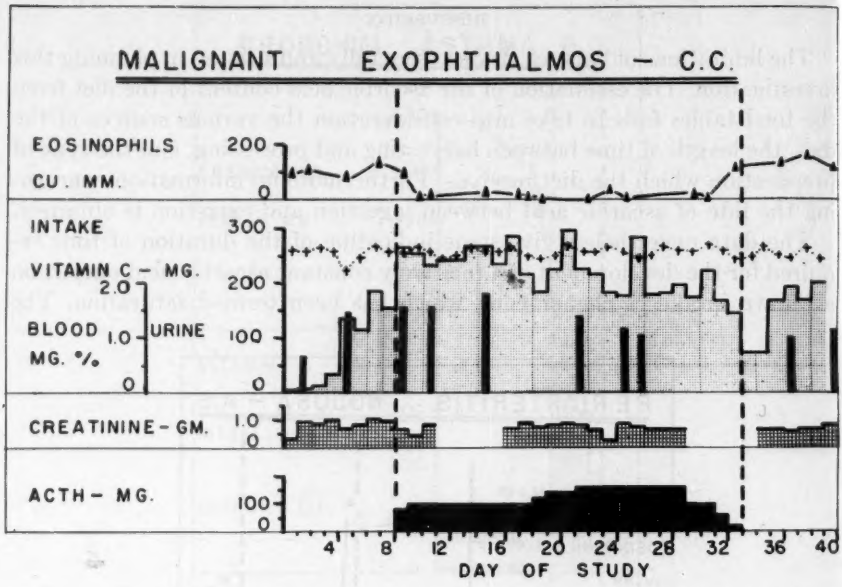

FIG. 3. *Case 13.* Malignant exophthalmos and hyperthyroidism controlled by propylthiouracil. Initially there was a very low ascorbic acid output and it required seven days on a known intake to reach a fairly constant urinary excretion. During daily administration of 100 mg. of ACTH there was an increase of 100 mg. per twenty-four hours in the ascorbic acid excretion. This increase persisted for twenty days, during part of which time the patient received 160 mg. of ACTH daily. Upon reduction in ACTH dosage a slow fall in excretion began, and on cessation of ACTH a sharp decrease in the urinary ascorbic acid excretion occurred, which persisted for two days.

time required in those subjects with a 24-hour ascorbic acid intake of approximately 250 mg. ranged between zero and twelve days. On an intake of 1,000 mg. there was immediate saturation. In the low ascorbic intake group the urinary output remained at a constant low level. These variations in the time taken to saturate the organism probably depend on the previous intake. Apparently the depleted tissue levels are replenished before increased urinary excretion occurs.

These studies also demonstrate that under normal conditions the excretion of ascorbic acid in the urine is less than the intake. The fate of a portion of the remainder has been clarified by recent work with C_{14} labelled ascorbic acid; part of the labelled carbon is found in the expired air as CO_2 (11, 12) and in the urine as calcium oxalate.

The results of this investigation are summarized in Table 2 and will be briefly discussed. Of the 27 patients receiving ACTH, 21 showed a sharp increase in the urinary ascorbic acid excretion during the first 24–48 hours

of ACTH administration, 3 showed a questionable increase, and 3 showed no alteration. In only a few patients was the increase maintained during the remaining period of ACTH injection. In general, those patients showing a peak in ascorbic acid excretion also displayed a definite, though variable, diminution in the urinary ascorbic acid excretion upon reduction or cessation of ACTH dosage. The dosage level of ACTH at which this occurred appeared to be approximately 50 mg. per twenty-four hours. It appears possible that the patients who showed no response to ACTH might have done so if the dosage had been sufficiently high.

Nine patients received intramuscular cortisone. Of these, 3 showed a definite increase in the urinary excretion of ascorbic acid, but in 6 no effect was noted. When an increase occurred it was step-like in character, suggesting that the cortisone level required to produce this phenomenon was reached slowly. This is in agreement with the observation that the microcrystals of cortisone acetate are more slowly absorbed when given intramuscularly.

The increase in urinary ascorbic acid excretion in the 3 patients receiving oral cortisone acetate suggests that the failure to respond to intramuscular cortisone was due to a difference in absorption rate and enables two

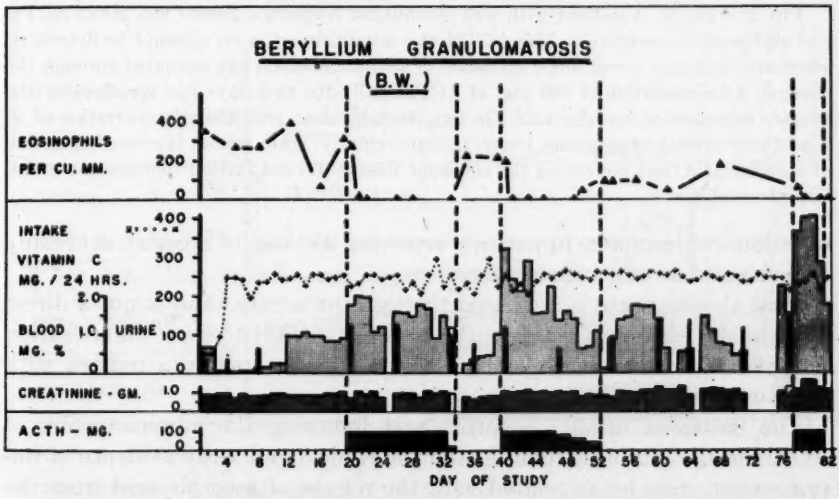

FIG. 4. *Case 14.* A patient with chronic berylliosis after seven days of known ascorbic acid intake excreted a constant amount of ascorbic acid in the urine. This patient received three courses of ACTH injections, each commencing with a 24-hour dosage of 100 mg. During each course there was a rise in the excretion of urinary ascorbic acid, amounting to approximately 100 mg. per twenty-four hours with the first course, 220 mg. with the second course, and 300 mg. with the third course. Upon cessation of ACTH administration there was a marked decrease in urinary ascorbic acid excretion.

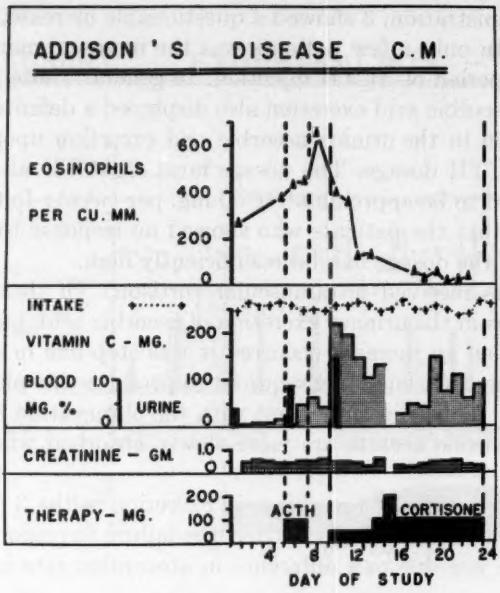

FIG. 5. *Case 19.* A patient with well established Addison's disease was given ACTH and cortisone consecutively. The ACTH was administered in an attempt to determine whether it had any direct effect on ascorbic acid metabolism not mediated through the adrenal. Administration of 100 mg. of ACTH daily for two days had no effect on the urinary excretion of ascorbic acid. On day 10, coincident with the administration of 50 mg. of cortisone in twenty-four hours, intramuscularly, there was an increased excretion of ascorbic acid; but increasing the cortisone dosage did not further increase the excretion of ascorbic acid.

questionable responses in patients receiving 250 mg. of ascorbic acid daily to be classed as positive responses.

That this response is mediated through the adrenal and is not a direct effect of ACTH is suggested by the failure of ACTH to cause an alteration in urinary ascorbic acid excretion when administered to a patient with Addison's disease.

The increased urinary ascorbic acid following the administration of ACTH in the scorbutic infants, and the clinical and x-ray evidence of improvement, may be associated with the release of ascorbic acid from the adrenal cortex in response to ACTH stimulation. Harris (13) has shown that in scurvy the adrenal is not completely depleted of ascorbic acid. This observation is in agreement with the favorable effects of adrenocortical extracts in experimental scurvy (14). However, the demonstration that oral cortisone can produce a similar clinical and biochemical effect suggests a common mechanism. It may be postulated that the adrenocortical hor-

mones either permit ascorbic acid to be synthesized in the human organism or that they enable the tissues to utilize substances which are chemically closely similar to ascorbic acid. The latter suggestion appears to be the less likely of the two, since ascorbic acid is detected in the urine; but this should be verified by biologic assay, as the small quantities present may be difficult to determine chemically.

At the present time it is only possible to offer a tentative explanation of the increased urinary ascorbic acid excretion seen in these studies. The available evidence suggests a renal mechanism as the most likely one. Ralli, Friedman and Rubin (15) have shown that the quantity of vitamin C excreted in the urine of an individual is determined by the plasma concentration, the glomerular filtration rate and the maximal rate of tubular reabsorption. They report the latter to be 2.16 mg. of ascorbic acid per minute, and when this was exceeded the excess vitamin present in the glomerular filtrate was excreted in the urine. Giroud and Leblond (16), using histologic techniques, have shown that this reabsorption occurred in the proximal convoluted tubule. ACTH has been reported to increase the glomerular

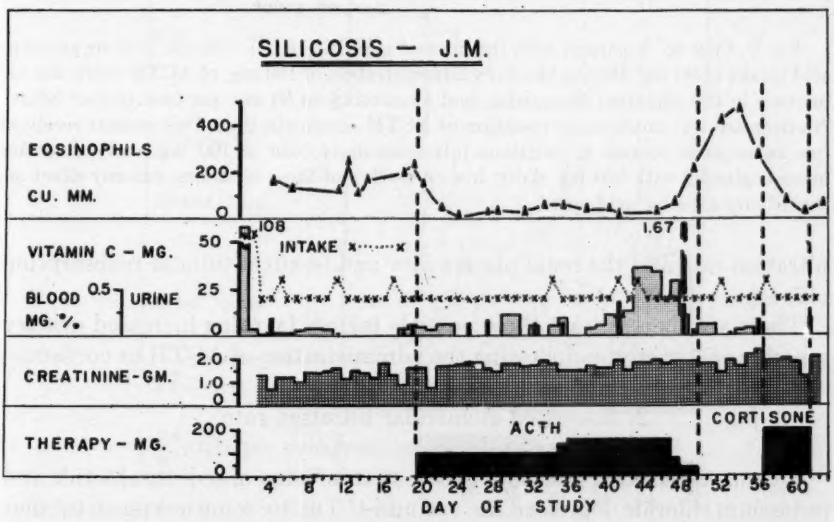

FIG. 6. *Case 22.* This patient with Grade III silicosis had an average 24-hour intake of 20 mg. of ascorbic acid. There was no increased urinary output with a daily dose of 100 mg. of ACTH, nor was there any apparent effect when the dosage was increased to 160 mg. However after eight days on this dosage there was an increase of 30 mg. per twenty-four hours in the urinary output of ascorbic acid. On reduction of the 24-hour ACTH dosage to 40 mg. there was a day during which no ascorbic acid could be detected in the urine. No increase in the ascorbic acid output occurred on the subsequent daily administration of 200 mg. of cortisone for five days.

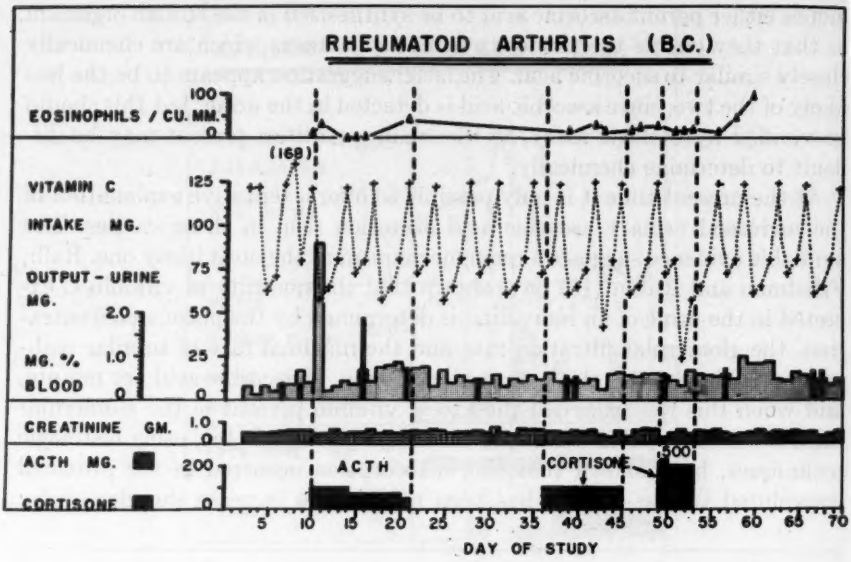

FIG. 7. *Case 25.* A patient with rheumatoid arthritis had an average 24-hour ascorbic acid intake of 90 mg. During the daily administration of 100 mg. of ACTH there was an increase in the excretion of ascorbic acid amounting to 80 mg. per twenty-four hours. No decrease was noted upon cessation of ACTH administration. This patient received two subsequent courses of cortisone intramuscularly, one of 100 mg. daily, and the other beginning with 500 mg. daily, but on neither of these occasions was any effect on the urinary ascorbic acid noted.

filtration rate and the renal plasma flow and to affect tubular reabsorption (17, 18).

There are then at least three possible factors favoring increased urinary ascorbic acid excretion following the administration of ACTH or cortisone:

1. The increased plasma level.
2. Increased glomerular filtration rate.
3. Lowered tubular reabsorptive capacity.

Selkurt and Houck (19) have shown that infusion of sodium chloride and potassium chloride depressed the vitamin-C Tm. by some unknown tubular mechanism, and it seems possible that alterations in electrolyte excretion associated with corticotropin and cortisone administration may play a role in increasing the urinary ascorbic acid. However, it is difficult to understand why the increase of ascorbic acid excretion does not always continue for the duration of ACTH administration, unless these renal alterations are temporary. The rise in the blood ascorbic acid level during the period of ACTH administration tends to rule out a renal mechanism as the only fac-

tor in the increased excretion. Renal clearance studies would be of great value in shedding further light on this aspect of the problem.

The possibility that the increased ascorbic acid excretion may be due to a rapid and continued release of this substance from the adrenal cortex must also be considered. The work of Sayers and associates (20) would tend to support this view. They observed a fall in the ascorbic acid and cholesterol content of the adrenals of rats after the injection of ACTH. The fall was maximal at one hour and persisted for two to three hours. It returned to its original level after twenty-four hours and finally reached a higher level than in the controls. This suggests that the adrenal cholesterol and ascorbic acid is intimately bound up with the synthesis of the cortical hormones. Giroud (21) has suggested that the synthesis of the cortical

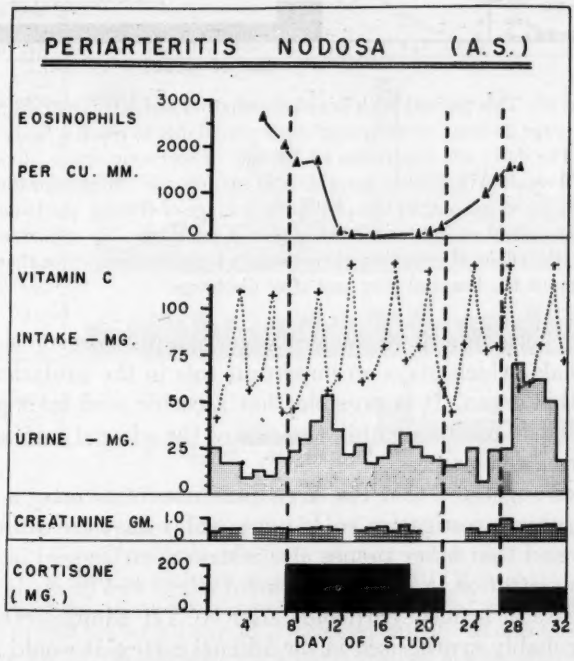

FIG. 8. *Case 27.* Periarteritis nodosa. Five months earlier this patient had a course of ACTH with a typical response. The average 24-hour ascorbic acid intake was 80 mg. and upon daily administration of 200 mg. of cortisone intramuscularly there was a step-like increase in the excretion of ascorbic acid, reaching a peak on the fourth day of administration. During the second course of cortisone administration there was again an increase in ascorbic acid output, this time reaching its peak on the second day of administration of 100 mg. of cortisone daily. Cessation of cortisone administration did not result in a decrease in the urinary ascorbic acid excretion.

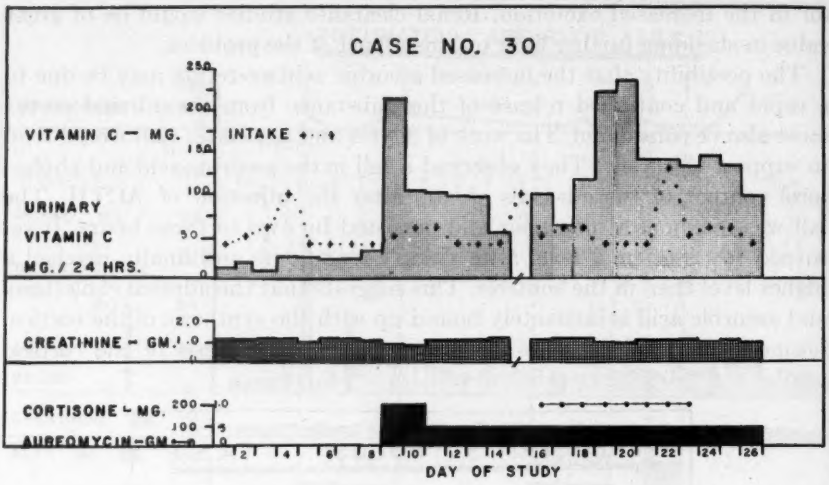

FIG. 9. *Case 30.* This patient with mixed rheumatoid and osteoarthritis required three days on an average 24-hour ascorbic acid intake of 50 mg. to reach a fairly constant urinary output. The daily administration of 200 mg. of cortisone orally (divided into four doses) on day 9 resulted in a rise of ascorbic acid excretion to 190 mg. for the first twenty-four hours. The increased output then fell to an average of 100 mg. per twenty-four hours in excess of the control period for the remainder of the study. No opportunity presented itself to study the effect of cessation of cortisone administration, since therapy was continued throughout the hospital stay and after discharge.

hormone, or hormones, is dependent upon the presence of ascorbic acid in the adrenals, which plays an important role in the oxidation-reduction process in this organ. It is possible that ascorbic acid facilitates certain types of chemical reaction within the cells of the adrenal cortex, and is discharged during this process.

It has been suggested that the large quantities of ascorbic acid found in the urine in this investigation could not possibly have the adrenal as their only source and that other tissues also release ascorbic acid in response to ACTH administration. A similar statement might also be made with regard to the increase in urinary corticoids after ACTH administration, though these are probably synthesized in the adrenal cortex. It would appear possible that ascorbic acid is also synthesized in the adrenal or withdrawn from other tissues and transported to the adrenal for a more vital function. Further investigations along the lines of obtaining adrenal blood after the administration of these hormones would further the understanding of this problem.

Finally, the source of the increased urinary ascorbic acid may be a general release of ascorbic acid from extra-adrenal tissues.

The foregoing is largely speculation and perhaps no one hypothesis fully answers the problem, since a combination of factors may be at work.

SUMMARY

1. The effect of ACTH and cortisone acetate on the blood and urinary levels of ascorbic acid was studied in 32 chronically diseased subjects.

2. Fifteen patients on an ascorbic acid intake of 250 mg. per twenty-four hours received ACTH. Twelve of these showed an increased urinary output of ascorbic acid, 2 showed a questionable increase, and 1 patient

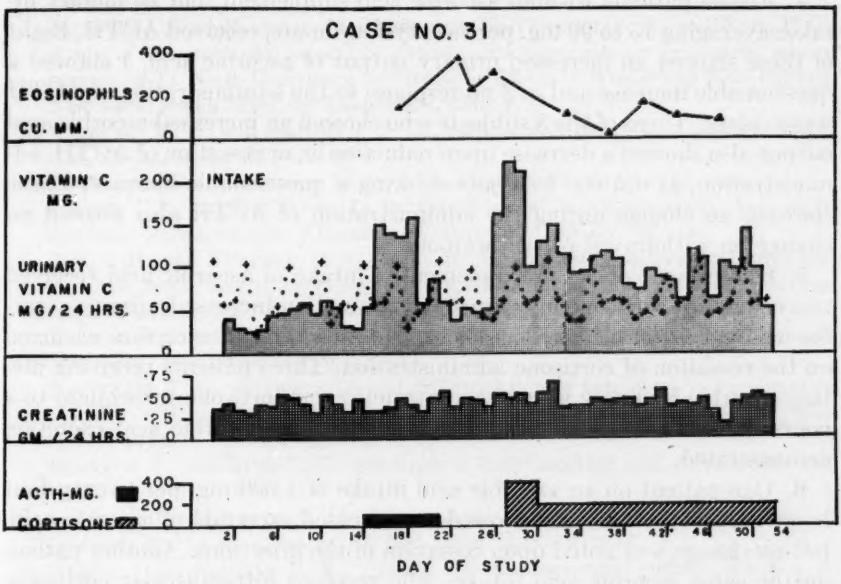

FIG. 10. *Case 31.* This patient with rheumatoid arthritis, on an average 24-hour ascorbic acid intake of 65 mg., reached a relatively constant output on the second day of the control period. The daily administration of 100 mg. of ACTH beginning on day 15 resulted in an increased ascorbic acid excretion, which reached a peak of 150 mg. per twenty-four hours on the second day. This increased output was maintained during the remaining period of ACTH injection, which was in contrast to a previous study in which the increase was temporary.

In a second control period (days 22–26) the average output was 50 mg. per twenty-four hours, and on the oral administration of 400 mg. of cortisone daily (divided into four doses) there was an increased ascorbic acid excretion, reaching a peak of 224.0 mg. during the second day. This was maintained at approximately 150 mg. per twenty-four hours in excess of the average control level for a subsequent twenty-five days. This subject had shown no response to two previous courses of cortisone given intramuscularly.

showed no change. Upon reduction of ACTH dosage below approximately 50 mg. per twenty-four hours, or upon the cessation of ACTH administration, 14 patients showed a decrease in the urinary ascorbic acid excretion which lasted from one to three days.

3. Four patients on an ascorbic acid intake of 250 mg. per twenty-four hours received intramuscular cortisone acetate. In 2 of these there was an increase in the urinary ascorbic acid excretion, and in 2 no effect on the urinary output could be demonstrated. No alteration in the ascorbic acid excretion occurred on cessation of cortisone administration in any of these cases.

4. Eleven patients without ascorbic acid supplement, but on dietary intakes averaging 15 to 90 mg. per twenty-four hours, received ACTH. Eight of these showed an increased urinary output of ascorbic acid, 1 showed a questionable increase and in 2 no response to the administration of ACTH was evident. Three of the 8 subjects who showed an increased ascorbic acid output also showed a decrease upon reduction in, or cessation of ACTH administration, as did the 1 subject showing a questionable increase. Those showing no change during the administration of ACTH also showed no change on withdrawal of the hormone.

5. One patient on an unsupplemented intake of ascorbic acid received two courses of cortisone injections and showed an increased urinary ascorbic acid excretion on both occasions. No alteration in excretion occurred on the cessation of cortisone administration. Three patients receiving dietary ascorbic acid only were given intramuscular cortisone subsequent to a course of ACTH, and in none was any effect on ascorbic acid excretion demonstrated.

6. One patient on an ascorbic acid intake of 1,000 mg. per twenty-four hours received ACTH and showed an increased excretion of ascorbic acid, but no change was noted upon cessation of the injections. Another patient on the same ascorbic acid intake, who received intramuscular cortisone, showed no alteration in ascorbic acid excretion either during or after the cessation of injections.

7. Two subjects on an unsupplemented ascorbic acid intake showed a marked and sustained increase in the excretion of ascorbic acid during the administration of oral cortisone.

8. Two scorbutic infants showed a rise in the blood and urinary ascorbic acid levels upon the injection of ACTH, and 1 upon the oral administration of cortisone. This was accompanied by a rise in the urinary formaldehydogenic corticoids and was associated with marked clinical improvement.

9. The blood ascorbic acid levels tend to rise at the same time that the urinary excretion of this substance is increased.

10. The possible sources of the increased ascorbic acid output and its implications in adrenocortical physiology are discussed.

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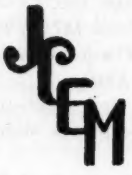

EFFECT OF ACTH ON THE ADRENALS IN THE NEPHROTIC SYNDROME AND RHEUMATIC FEVER*

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INTRODUCTION

A PREVIOUS paper has presented the results of an analysis of the effects of adrenocorticotropic hormone (ACTH) and compound E (cortisone) on the adrenals and anterior pituitaries of children with acute leukemia (1). Although a considerably smaller amount of material was available, the results of a study of the effects of ACTH on the adrenals of patients with the nephrotic syndrome due to chronic glomerulonephritis and with rheumatic fever seemed of sufficient interest to merit a brief report.

MATERIAL AND METHODS

The adrenal tissue was obtained, prepared, and studied in the same way as the material from the patients with acute leukemia described in the earlier paper (1). Table 1 shows the age and sex distribution of the patients studied, all of whom were treated by the medical service of The Children's Medical Center, or by the staff of the House of the Good Samaritan. Tables 2 to 5 show data concerning the adrenals of these patients.

RESULTS

The adrenals of the control patients with the nephrotic syndrome had the smallest average weights, in terms of the standard values, of any group in our entire study. The cell fat was higher in all three zones than it

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TABLE 1. AGE AND SEX OF PATIENTS WITH 1) THE NEPHROTIC SYNDROME DUE TO CHRONIC GLOMERULONEPHRITIS AND 2) RHEUMATIC FEVER

Age (yrs.)	Patients who had the nephrotic syndrome				Patients who had rheumatic fever			
	Control		Treated		Control		Treated	
	Male	Female	Male	Female	Male	Female	Male	Female
0–2	1							
3–5	1		4	1	1			
6–10	1				2	1	1	1
11–20								4
More than 20								2
Total	3	0	4	1	3	1	1	7

TABLE 2. DATA CONCERNING PATIENTS WITH THE NEPHROTIC SYNDROME AND WITH RHEUMATIC FEVER, TREATED WITH ACTH AND CORTISONE

Group	No. of cases in which adrenals were exam'd.	Avge. total dose (mg.)	Weight of adrenals (% of standard)
1. Patients with the nephrotic syndrome not treated with ACTH (controls)	3		117
2. Patients with the nephrotic syndrome who died while being treated with ACTH	1	900	376
3. Patients with the nephrotic syndrome who died after having being treated with ACTH	4	913	190
4. Patients with rheumatic fever not treated with ACTH or cortisone (controls)	3		142
5. Patients with rheumatic fever who died while being treated with ACTH	4	374	134
6. Patients with rheumatic fever who died while being treated with ACTH and cortisone	1	ACTH 140 Cortisone 400	175
7. Patients with rheumatic fever who died after having been treated with ACTH	4	491	167

TABLE 3. RESULTS OF HEMATOXYLIN AND EOSIN STAIN ON ADRENALS FROM CHILDREN WITH THE NEPHROTIC SYNDROME AND WITH RHEUMATIC FEVER, TREATED WITH ACTH AND CORTISONE

Group	Zona glomerulosa								
	Thickness			Cell size			Cell fat		
	Thin	Normal	Thick	Small	Normal	Large	Low	Normal	High
1	33	33	33	66	0	33	0	33	66
2	0	0	100	0	0	100	100	0	0
3	0	75	25	25	75	0	25	50	25
4	0	33	66	0	100	0	66	33	0
5	75	25	0	0	100	0	50	50	0
6	100	0	0	100	0	0	100	0	0
7	0	75	25	0	100	0	50	25	25

Group	Zona fasciculata								
	Thickness			Cell size			Cell fat		
	Thin	Normal	Thick	Small	Normal	Large	Low	Normal	High
1	0	66	33	0	66	33	33	33	33
2	0	0	100	0	0	100	100	0	0
3	0	75	25	0	100	0	50	25	25
4	33	33	33	0	100	0	33	66	0
5	0	75	25	0	100	0	50	50	0
6	0	100	0	0	0	100	100	0	0
7	25	75	0	0	75	25	75	0	25

Group	Zona reticularis								
	Thickness			Cell size			Cell fat		
	Thin	Normal	Thick	Small	Normal	Large	Low	Normal	High
1	0	100	0	0	33	66	33	33	33
2	0	0	100	0	0	100	100	0	0
3	0	75	25	0	100	0	50	25	25
4	66	33	0	0	100	0	33	66	0
5	0	50	50	0	100	0	50	50	0
6	0	100	0	0	100	0	100	0	0
7	25	75	0	0	75	25	75	0	25

was in the leukemic or rheumatic controls, particularly in the zona glomerulosa, but the other histologic features were not strikingly different from those of the other control groups. Only 1 nephrotic patient died during administration of ACTH; this patient's adrenals showed the typical increase in weight, increase in thickness and cell size of the zona fasciculata and zona reticularis, and decrease in cell fat in these two zones. In addition, the zona glomerulosa showed a similar increase in zone thickness and cell size and a similar decrease in fat. After cessation of ACTH therapy, the adrenals of the nephrotic patients showed a return to values not significantly different from those of the controls.

The control adrenals from patients with reumatic fever had an average

TABLE 4. RESULTS OF SUDAN-4 STAIN ON ADRENALS FROM CHILDREN WITH THE NEPHROTIC SYNDROME AND WITH RHEUMATIC FEVER, TREATED WITH ACTH AND CORTISONE

Group	Zona glomerulosa					Zona fasciculata					Zona reticularis				
	None	Doubtful	Weak	Moderate	Strong	None	Doubtful	Weak	Moderate	Strong	None	Doubtful	Weak	Moderate	Strong
1	0	0	0	0	100	0	33	33	33	0	0	66	23	0	0
2	100	0	0	0	0	0	0	100	0	0	0	0	100	0	0
3	0	25	75	0	0	0	0	100	0	0	0	0	100	0	0
4	25	25	25	0	25	0	0	75	25	0	0	50	25	25	0
5	50	0	25	0	25	50	0	25	0	25	0	50	50	0	0
6	0	0	0	100	0	0	100	0	0	0	0	0	100	0	0
7	25	50	25	0	0	0	0	75	0	25	0	25	50	0	25

TABLE 5. RESULTS OF ASHBEL-SELIGMAN REACTION ON ADRENALS FROM CHILDREN WITH THE NEPHROTIC SYNDROME AND WITH RHEUMATIC FEVER, TREATED WITH ACTH AND CORTISONE

Group	Zona glomerulosa			Zona fasciculata			Zona reticularis		
	None	Doubtful	Weak	None	Doubtful	Weak	None	Doubtful	Weak
1	66	33	0	66	33	0	66	33	0
2	100	0	0	100	0	0	0	100	0
3	50	0	50	50	0	50	25	0	75
4	75	25	0	100	0	0	75	25	0
5	25	75	0	25	75	0	25	75	0
6	0	0	100	0	0	100	0	100	0
7	50	25	25	50	0	50	25	75	0

weight lower than that for the control leukemic adrenals, but somewhat higher than that for the adrenals from controls with the nephrotic syndrome. In the patients who died while being treated with ACTH, the adrenals did not increase in size, and the various cortical layers showed no significant change in the hematoxylin and eosin stained preparations. The average total dose of ACTH received by this group was only 374 mg. The fat stainable by Sudan 4 probably decreased in the zona fasciculata only. The adrenals of the one patient who died while being treated with both ACTH and cortisone showed a weak ACTH effect. The adrenals of the patients with rheumatic fever who died after cessation of ACTH therapy did not differ from those of the untreated controls.

DISCUSSION

The small size and relatively higher fat content of the adrenals of patients with the nephrotic syndrome suggest the effect of cortisone (1), or of hypophysectomy (2). Whether these adrenals show the effect of abnormally low endogenous ACTH production, or whether the control patients with leukemia and perhaps also those with rheumatic fever have increased endogenous ACTH production, cannot be stated. The data do not allow an estimate as to the role of altered sensitivity of the adrenal to ACTH in any of these conditions.

Microscopically, there would seem to be no apparent component of adrenocortical overactivity in the salt and water retention aspect of the nephrotic syndrome. The response of the zona glomerulosa to ACTH in the nephrotic syndrome, if confirmed by further studies, is similar to that described in a previous paper (1) for the zona glomerulosa of patients with acute leukemia treated with cortisone before receiving ACTH; whether the mechanism is the same cannot be stated. The observations on patients who had rheumatic fever support the suggestion made for those with leukemia, that the zona fasciculata is more responsive to ACTH than the zona reticularis, and the findings in the one patient with rheumatic fever who died while being treated with both ACTH and cortisone are similar to those of the corresponding leukemic patients; namely, the adrenal fat and the adrenal weight both increased. Again, this suggests that cortisone may have some direct effect on the adrenal cortex in addition to its ability to depress ACTH production.

SUMMARY

The effects of ACTH on the adrenals of small groups of patients with the nephrotic syndrome due to chronic glomerulonephritis and with rheumatic fever are presented. The adrenals of control untreated patients with the nephrotic syndrome had a smaller average weight and a higher fat

content than those of untreated patients who had rheumatic fever or leukemia. In the one nephrotic patient who died while being treated with ACTH, all three zones of the adrenal cortex showed a decrease in fat and an increase in zone thickness and cell size, the response of the zona glomerulosa being similar to that seen in leukemic patients treated with cortisone before receiving ACTH. The findings in the patients with rheumatic fever support those seen in patients with leukemia, namely, that the zona fasciculata is more responsive to ACTH than is the zona reticularis, and that cortisone alters the morphologic response of the adrenal cortex to ACTH.

Acknowledgment

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STUDY OF THE EXTRATHYROIDAL EFFECTS OF THYROID-STIMULATING HORMONE*†

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OF THE major body constituents, fat has received the least attention, primarily because of lack of precise methods for its measurement. However, there has been considerable fundamental investigation related to body composition which suggests the possibility of indirectly estimating the fat content of the body. In 1942 Behnke and his associates secured evidence from specific gravity measurements in human subjects which indicate that fat, because of its comparatively low specific gravity, is the major determinant of corporeal specific gravity (1). In 1945 Rathbun, Pace, Morales and Smith provided data, based on a study of small animals, which indicate that measurement of the body specific gravity permits estimation of the relative amount of fat in the body (2, 3, 4). Recent studies have served to strengthen the belief that measurement of body specific gravity is a cumbersome yet suitable method whereby total body fat can be estimated (5, 6). The present study is a portion of continuing investigation underway in these laboratories and directed toward utilization of measurement of body specific gravity for the evaluation of changes in body composition which result from various hormonal influences.

A number of extrathyroidal effects, especially pertaining to fat, have been attributed to the pituitary thyroid-stimulating hormone (TSH). Consequently, it seemed reasonable to apply this technic for estimation of body composition, together with other metabolic measurements, to determine whether TSH exerts any significant effect in the absence of functioning thyroid tissue in the human.

MATERIALS AND METHODS

Two patients with myxedema were selected for study and hospitalized

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‡ William Wade Hinshaw Cancer Research Fellow.

after thyroid medication had been discontinued for approximately eight weeks.

Weights were obtained each morning before breakfast and following completion of the 24-hour period of urine collection. Food intake was maintained at a constant level with respect to calories and nitrogen, as calculated from standard food tables. Rejected food was given with the following meal, unfinished portions of the evening meal being given before sleep. Water balance was calculated from the difference between liquid intake and urinary output. Blood for laboratory studies was drawn in relatively constant amounts at regular intervals so as to permit estimation of the average nitrogen loss by this route. Urinary nitrogen was measured by a micro-Kjeldahl technic; fecal nitrogen excretion was extimated as 1.3 Gm. per day. Blood and urine sodium and potassium levels were measured by the flame photometer. Total blood lipids were measured by the method of Bloor (7), lipid phosphorus by that of Youngburg and Youngburg (8), and cholesterol by the chromatographic technic of Hess (9). Blood ketones were measured by the method of Michaels and associates (10), pyruvic acid by that of Lu (11) and amino nitrogen by that of Frame, Russell and Wilhelmi (12).

Body specific gravity was obtained by the method[1] of underwater weighing, described by Behnke, Feen and Welham (1). The application of this technic to the study of changes in body fat in humans has been previously reported from these laboratories (13, 14). Total body fat was calculated by the Rathbun-Pace equation (2), which is formulated on the basis that the specific gravity of fat is 0.918.

$$\% \text{ Fat} = 100\left(\frac{5.548}{\text{sp.gr.}} - 5.044\right)$$

Body water was calculated from the specific gravity by substitution in the equation derived by Ossermann (15):

$$\% \text{ Body water} = 100\left(4.340 - \frac{3.983}{\text{sp.gr.}}\right)$$

Fat-free solids were assumed to be the difference between total body weight and the sum of the weight of fat and water. Residual air was measured successively during the study by the helium dilution technic of Willmon and Behnke (16). At the time of each measurement, weights were obtained at both full inspiration and deep expiration so as to be certain that the subject was expiring to a relatively constant volume of residual air.

RESULTS

The first subject was a 22-year-old white man who had been taking thyroid hormone regularly since early childhood. Because of his tolerance to large amounts of this drug (about 300 mg. per day) the presence of true

[1] Specific gravity was calculated from the formula:

$$\text{Sp. gr.} = \frac{\text{wt. in air}}{\text{wt. in air} - \text{true wt. in water}},$$

submersed weight being measured at a water temperature of 32–34° C. In correcting for residual lung volume no allowance was made for the water density because of the very minor error involved.

thyroid deficiency was questioned. Although the typical changes of thyroid deficiency appeared rapidly after suspension of therapy, it seemed desirable to attempt stimulation of the thyroid gland with thyroid-stimulating hormone (TSH).

After suitable control studies, including 24-hour uptake of I^{131} and repeated BMR determinations, thyrotropic hormone[2] was given for twenty-two days in daily dosages of 25 mg., 37.5 mg. and finally 50 mg. (in terms of

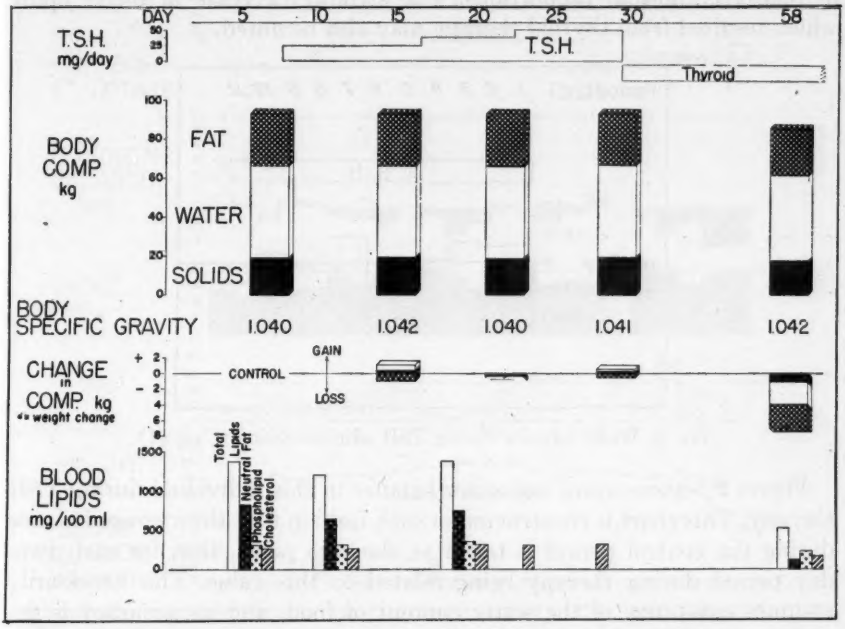

FIG. 1. Changes in body composition and blood lipids during administration of TSH and thyroid hormone (Case #1).

the Armour House standard) divided into two or three daily doses. During the period of treatment body specific gravity showed no significant change and, as body weight remained constant, it is apparent that the gross body composition was unaffected. In Figure 1 the estimated composition is illustrated by columns, and the changes in fat, water and fat-free solids on the scale below. Total blood lipids, partitioned into neutral fat, phospholipids and cholesterol did not vary significantly during treatment and there was no demonstrable change in the blood levels of pyruvic acid or amino

[2] Thyrotropic hormone used for this study was generously furnished by the Armour Laboratories.

nitrogen. The BMR remained depressed and the 24-hour uptake of radioiodine was less than 1 per cent, both before and at the termination of TSH therapy.

When desiccated thyroid was administered, definite changes occurred. However, despite the loss of nearly 7 Kg. of weight during the first month of thyroid treatment the body specific gravity did not change materially, indicating that there had occurred loss of all three major body constituents in relatively normal proportions. The striking decrease in blood lipids which resulted from thyroid therapy may also be noted.

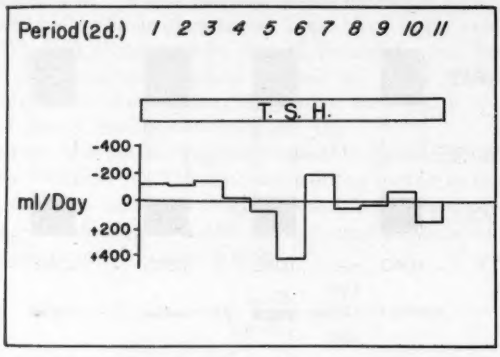

FIG. 2. Water balance during TSH administration (Case #1).

Figure 2 demonstrates the water balance in this individual during TSH therapy. This chart is constructed in such fashion that the average balance during the control period is taken as the zero point, that for each two-day period during therapy being related to this value. This necessarily assumes constancy of the water content of food, and its accuracy is dependent upon a constant insensible water loss. The apparent validity of these assumptions is seen by the fact that water balance did not vary significantly during twenty-two days of TSH injection.

In Figure 3 is shown the result of administration of larger amounts of TSH for a total of twenty-four days in the second experimental subject. In this 48-year-old man the typical features of thyroid deficiency developed several years after subtotal thyroidectomy for a diffuse toxic goiter. In this instance, whereas the 24-hour uptake of radioiodine was 10 per cent prior to administration of TSH, it was only 2 per cent following the termination of therapy and there occurred no increase in basal oxygen consumption. In Figure 3, nitrogen excretion is charted after the method of Moore (17), with correction made for blood sampling and constant fecal excretion. There occurred a moderate increase in nitrogen excretion in the first half of the treatment period, as well as a questionably significant increase in

urinary potassium excretion. However, these minimal changes, together with the more marked decrease in urinary sodium excretion, coincided with an intense inflammatory reaction at the site of the gluteal injections of TSH. When sites of injections were rotated, the reactions disappeared and excretion of potassium, sodium and nitrogen returned to the control levels. As in the first subject, there was no change in blood levels of pyruvic

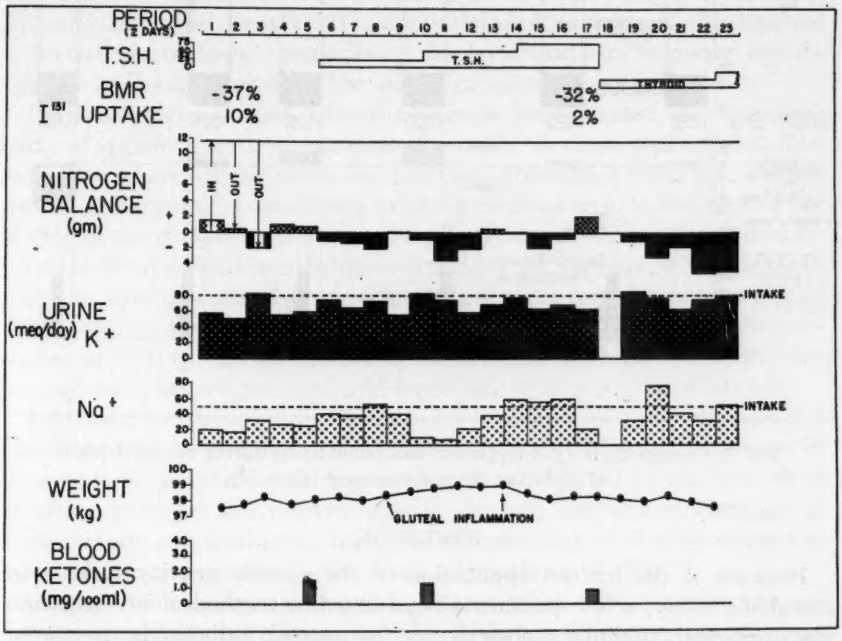

FIG. 3. Metabolic changes during administration of TSH and thyroid hormone (Case #2).

acid or amino nitrogen, and blood ketone levels were not elevated during the administration of TSH.

Figure 4 shows the body composition measurements and the blood lipid concentrations in this subject. As in the first patient, there was no significant change in body specific gravity and, as body weight remained constant, gross body composition was apparently unaffected. Lack of significant loss or gain in fat, water or fat-free solids is clearly shown, as well as the constancy of the blood lipid concentration. After thirty-six days of thyroid therapy, definite loss of weight and decrease in blood lipids were apparent. Although there appeared to be some loss of all major body components, fat loss predominated in this individual.

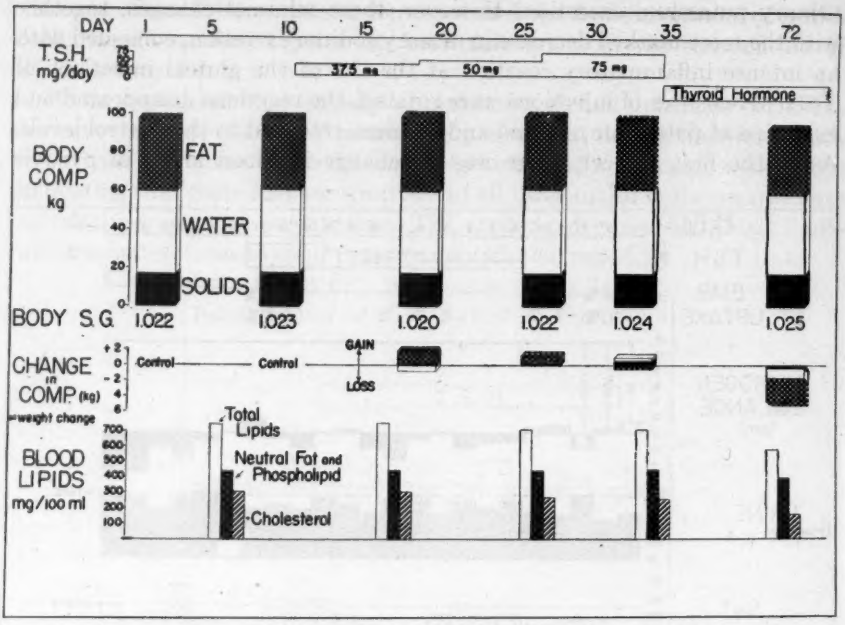

FIG. 4. Changes in body composition and blood lipids during administration of TSH and thyroid hormone (Case #2).

DISCUSSION

Because of the limited application of the specific gravity technic to metabolic study, a few comments regarding this method of investigation are applicable. Behnke's observation, in normal individuals, indicated that the body is composed of a lean mass of relatively constant composition plus a varying amount of fat. Experimental data from which formulae for calculation of body fat and water are derived, were secured by Rathbun, Pace and Morales from measurement of small animals. Although these animals have a proportionately lesser skeletal mass than the human subject, the data appear to be valid when applied to humans (18). Calculation of total amounts of fat, water and fat-free solids is quantitatively valid, however, only under conditions of normal body composition and equal precision cannot be expected in the presence of abnormal states. Thus the graphic dissection of these 2 myxedematous subjects into the three major body components, as shown in Figures 1 and 4, is illustrative rather than real; for it may be assumed, considering the retarded tissue turnover and fluid accumulation of myxedema, that these components were not in normal proportions. On the other hand, the marked difference in the specific

gravity of the three components, *i.e.*, 0.930, 0.994 and 1.40 for adipose tissue,[3] water and tissue solids, respectively, would necessarily require that any material loss or gain of any substance would be accompanied by a significant change in body specific gravity (19). Even with measurement of specific gravity alone, the vector of change would be apparent; with concurrent measurement of nitrogen or water balance, tissue change would be more precisely defined. Recent reviews by Behnke, Osserman, and Welham (20) and Keys and Brozek (21) supply detailed analyses of this method of estimation of body composition, the latter authors stressing certain fallacies in the application of the densitometric technic.

Previous studies in these laboratories have demonstrated the reproducibility of specific gravity measurement even in the abnormal subject. The data shown here further illustrate that fact. Although a variation of 0.003 specific gravity unit is considered to be the minimal error in the application of this measurement to healthy young individuals, serial determinations in these 2 myxedematous subjects during TSH therapy fell within the limits of this theoretical error. Thus, these data indicate not only that there occurred no gross changes in body composition during the administration of TSH to athyrotic patients, but also that accurate specific gravity measurement is both practical and reproducible in this type of subject.

Collateral metabolic studies indicated that neither nitrogen excretion nor water balance were significantly affected by TSH. Absence of change in intermediary metabolism during treatment is indicated by the constancy of amino nitrogen and pyruvic acid levels; and the lack of variation in blood ketone and lipid levels serves to indicate that fat is not undergoing mobilization or oxidation as the result of injections of TSH.

Over a number of years, frequent reference has been made to the apparent extrathyroidal effects of TSH. Although the inception of these views was probably based on our inability to explain either progressive exophthalmos or local myxedema on the basis of increased production of thyroid hormone, a certain amount of animal experimentation has indicated that pituitary extracts may cause tissue changes unrelated to their stimulating effect on the thyroid gland. Smelser demonstrated infiltration of both lymphocytes and a stainable material into the extraocular muscles and orbital fat of guinea pigs treated with TSH (22). Paulson confirmed certain of these observations and found muscle degeneration and round-cell infiltration in cardiac and skeletal muscles after TSH injections, regardless of the presence or absence of the thyroid gland (23). Pochin and Rundle found a marked increase in adipose tissue between the fibers of the skeletal

[3] It may be noted that the specific gravity value for adipose tissue is considered to be greater than that for fat, because "fatty tissue" contains both fat and protein.

eye muscles in patients with thyrotoxicosis (24). Turner reported that the administration of TSH to hypercholesteremic rabbits produced a fall in blood cholesterol regardless of the presence or absence of the thyroid gland (25). Pugsley, however, was unable to demonstrate any effect of TSH on blood cholesterol in the thyroidectomized dog (26).

Dobyns has made notable contributions to the related subjects of exophthalmos and thyrotropic hormone activity (27). Study of the effects of Antuitrin-T in animals showed an increase in round cells containing many fine droplets of fat in orbital tissue, fat depots and, to lesser degree, in muscle. Muscle fibers throughout the body, as well as cells of the liver and kidney, were loaded with fat droplets. Further studies of fat depots showed depletion of fat with replacement by a translucent gelatinous material associated with infiltration of neutrophils and tissue macrophages. The cellular reaction, as well as exophthalmos, occurred regardless of the presence or absence of the thyroid gland or testes. In comparing the effects of Antuitrin-T, crude TSH and purified TSH, Dobyns secured data which indicated that there occurred both a lesser exophthalmic effect and absence of change in fat with the more purified preparation. Jeffries has studied the effect of various doses of pituitary extract on the mobilization of fat and the production of exophthalmos in intact and thyroidectomized guinea pigs (28, 29). No evidence was obtained that either the exophthalmic or fat-mobilizing effects of pituitary extract could be enhanced by thyroidectomy and it appeared unlikely that the exophthalmic effect was due to changes in fat. Iodination of TSH appeared to decrease the thyrotropic effects out of proportion to the decrease in exophthalmic activity.

Although these studies serve to indicate that certain pituitary extracts affect both the eyes and tissue metabolism, even in the absence of the thyroid gland, investigation of the 2 subjects reported here demonstrates, at least in the human, no such effects following the injection of purified TSH. That this discrepancy is due, not to a species difference but to the nature of the hormone administered, is suggested by the apparent lesser degree of extrathyroidal effect induced by purer forms of TSH. This belief has been recently strengthened by further studies of Dobyns and Steelman, which indicate that there exists a pituitary exophthalmos-producing factor entirely distinct from the tropic hormone which stimulates the thyroid gland (30). It would therefore appear that TSH exerts its metabolic effects only through stimulation of the thyroid gland.

SUMMARY

Estimation of gross body composition by the specific gravity technic appears to be clinically feasible as an adjunct to other methods of metabolic study. This method of investigation appears to be suitable for measurement of gross change in body fat.

In 2 patients with myxedema due to thyroid deficiency, the administration of large amounts of TSH for twenty-two and twenty-four days, respectively, did not result in any change in body composition detectable through measurement of body specific gravity. Additional metabolic studies, including the concentration of blood lipids and ketones, also failed to document any metabolic effects of TSH. Definite changes in body weight and blood lipid concentration resulted from the administration of thyroid hormone.

It is concluded that TSH exerts a significant metabolic effect in the human subject only when functioning thyroid tissue is present.

Acknowledgment

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CONVULSIVE SEIZURES COMPLICATING CORTISONE AND ACTH THERAPY: CLINICAL AND ELECTRO-ENCEPHALOGRAPHIC OBSERVATIONS

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INTRODUCTION

As a result of the current extensive research with ACTH and cortisone and their clinical applications, there has been an increasing number of reports that these substances exert a profound influence on the electrical activity of the brain. Hoefer and Glaser (1) reported in 1949 that these hormones increase electroencephalographic abnormalities, whereas Thorn (2), Pine *et al.* (3), and Friedlander and Rottgers (4) came to opposite conclusions, namely, that ACTH and cortisone tend to produce normalization of the brain-wave patterns and an increase of alpha activity.

Convulsive seizures have occasionally been listed among the complications of cortisone and ACTH therapy. For instance, Dorfman and his co-workers (6) observed 3 cases of status epilepticus during the use of pituitary-adrenocortical hormones. Soffer, Baer and their co-workers (7) reported convulsions in 4 of 17 patients with lupus erythematosus early in the course of treatment with ACTH and cortisone. In experimental ACTH studies on 8 depressed patients Cleghorn (8) noted a series of convulsions in 1 subject.

Our own investigations (5) based on systematic evaluation of 43 patients without obvious metabolic, endocrine or emotional disorders, who had serial electroencephalograms before, during, and after ACTH and cortisone therapy, show that the stability of the electrical activity of the cortex prior to hormone therapy, is an important factor in evaluating the results of treatment on the EEG. Patients with initially normal or borderline EEG records showed relatively few or no changes during hormone therapy; but patients with moderately or grossly pathologic brain-wave patterns usually manifested a tendency to an increase in the defect and occasionally a development of patterns compatible with those seen in convulsive disorders. In 4 of these 43 patients clinical seizures were observed during or shortly after treatment with cortisone and ACTH. This led to an investigation of the potential convulsive properties of these hormones in the 4 cases mentioned.

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REPORT OF CASES

Case 1. S. K., a 32-year-old man, suffered from severe rheumatoid arthritis involving practically all major joints of the extremities and spine, and including ankylosing deformities. After a thorough clinical, radiologic, and laboratory work-up, the patient was started on cortisone (average dose, 100 mg. daily), to which he responded with some subjective improvement, associated with euphoria and water retention with rounding of the face and periorbital edema.

The EEG, grossly abnormal before therapy (Fig. 1), became increasingly pathologic and finally compatible with seizure patterns. An electrocardiogram obtained two weeks after the beginning of cortisone therapy showed changes strongly suggestive of hypopotassemia. Since the patient could not be safely maintained on the large doses of cortisone necessary to keep him comfortable, treatment was tapered off and discontinued after one month. During this time the patient had what were apparently minor clinical seizures, which had escaped the attention of the medical and nursing staff and were reported by the patient only in retrospect. These he called "muscle spasms," from which he had previously suffered. That this was a misidentification on the part of the patient became clear after he came under neuropsychiatric observation with a full-blown toxic psychosis. In the course of the psychiatric interview three convulsive episodes were observed within half an hour, each lasting from twenty to thirty seconds. They consisted of body jerking, twitching of the mouth, blinking of the eyes, momentary loss of consciousness, and amnesia for the episode. Subsequently the patient complained of headache. The EEG showed almost continuous seizure patterns, and many 2–3 per second high-voltage dome-shaped wave forms in the forward areas.

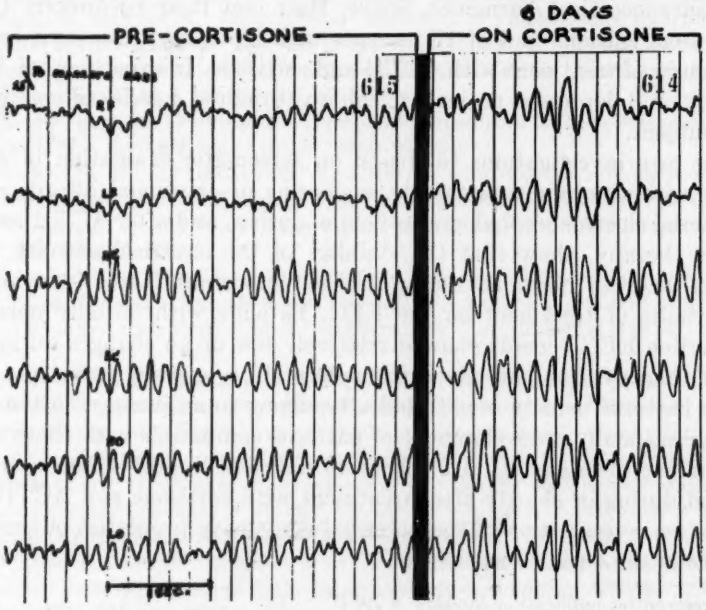

FIG. 1. *Case 1.* Pre-cortisone: EEG diffusely abnormal, generally slow patterns. During six days of cortisone therapy: EEG more grossly abnormal than prior to cortisone therapy.

Following induction of anticonvulsive medication [Tridione (trimethadione), Dilantin (diphenylhydantoin), or phenobarbital] the patient improved clinically (Fig. 2); Tridione, however, produced most improvement in the brain-wave patterns (Fig. 3). The psychosis cleared within two or three weeks. The patient was then able to relate that although he had frequent muscle spasms of the extremities prior to cortisone therapy, he became aware of a second type of "jolts" while he was taking cortisone. These were occasionally accompanied by momentary "blanks" followed by a "headachy" feeling, and were undoubtedly identical with the seizures observed during the psychiatric consultation just described. They promptly disappeared after initiation of anticonvulsive therapy. In view of past experience it was felt that further cortisone therapy would be without benefit, if not actually hazardous, to the patient. During the rest of his hospitalization he received maintenance doses of phenobarbital.

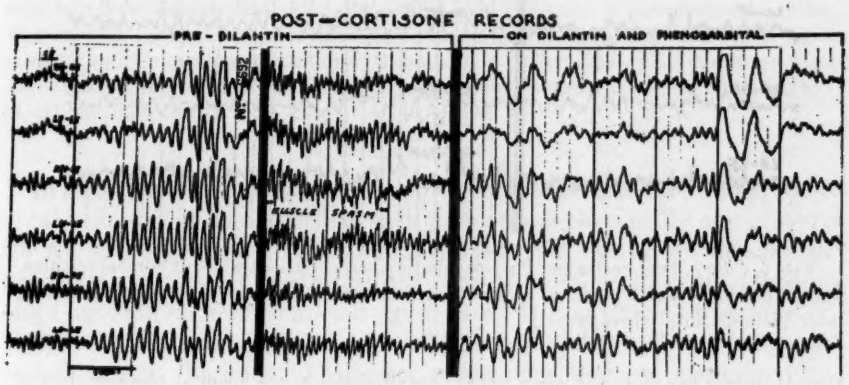

FIG. 2. *Case 1.* Post-cortisone: Patient psychotic—clinical epileptiform seizures. EEG is grossly and diffusely pathologic and shows extensive seizure activity. After initiation of anticonvulsive therapy, the forward areas remained abnormal longer than the posterior regions of the cortex.

Case 2. P. A. was a 27-year-old male with rapidly progressing lupus erythematosus. Seriously ill throughout his hospitalization, he responded to varying amounts of cortisone and ACTH with transient improvement.

Transitory psychosis developed six weeks after the beginning of cortisone therapy; three days later he had a short epileptiform episode for the first time, manifested by jerking movements of the jaw, twitching of the eyes, and momentary loss of consciousness. Prior to therapy his brain-waves had been pathologic—slow with paroxysmal features—and they remained so while he was taking cortisone. There was a greater build-up in the brain-wave patterns during hyperventilation while he was undergoing treatment than before. Otherwise, there was no evidence of metabolic disturbance. A second seizure occurred about six months later, ushering in the final deterioration in the patient's physical condition. Cortisone and ACTH therapy became ineffective, and he died within approximately three weeks. A postmortem examination showed no evidence of definite cerebral structural changes.[1]

Case 3. H. S. was a 36-year-old woman, who suffered from disseminated lupus ery-

[1] Report of postmortem examination of the central nervous system by Dr. Abner Wolf, Columbia University, College of Physicians and Surgeons.

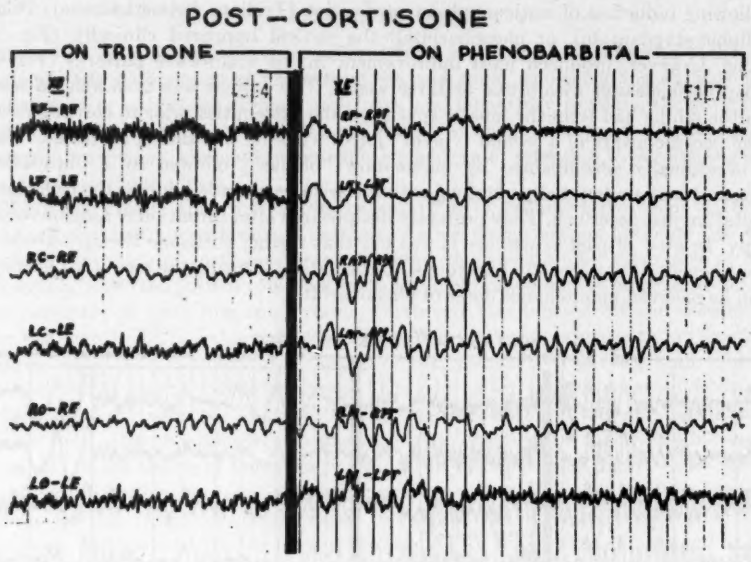

FIG. 3. *Case 1.* Post-cortisone: Post-psychotic phase—patient without clinical seizures. Improvement of EEG patterns is more marked during Tridione therapy than during later phenobarbital therapy.

thematosus for two and a half years prior to her admission to the hospital. She also showed signs of rheumatoid arthritis. For ten months before her admission she had received ACTH and cortisone elsewhere with benefit, and therefore was re-started on cortisone, 100 mg. per day, immediately after admission.

Pre-therapy EEGs were not available. However, the patterns were abnormal while the patient was receiving cortisone. About four months after admission to the hospital, *i.e.*, fourteen months after the beginning of cortisone and ACTH therapy, status epilepticus suddenly developed, preceded by emotional irritability and mounting fear. The first seizure was a major tonic-clonic convulsion, lasting approximately three minutes, followed by post-ictal confusion and lethargy. Within the following eight hours the patient had 14 additional major and minor convulsions despite large doses of anticonvulsive medication, spinal tap (s.p. 160 mm.), and omission of ACTH and cortisone. On that day blood chemistry showed a low potassium level (2.1 mEq./L.) and a rise in the sodium level to 145 mEq./L. The patient remained gravely ill, confused, and lethargic for two days subsequently, during which time she received large doses of anticonvulsants and potassium. On the third day dramatic improvement took place—the temperature fell to normal and the patient became alert but euphoric; however, there was some aphasia, which took from four to six weeks to disappear. Since she had been too ill to be moved to the EEG laboratory during and immediately following status epilepticus, tracings were not obtained until the third day after the first episode. At that time they were grossly abnormal, but showed improvement subsequently, although they always remained mildly abnormal even while the patient received Dilantin and phenobarbital. ACTH therapy was subsequently resumed because of an exacerbation of the lupus. She

had no further convulsions, and four months later was allowed to go home on a maintenance dose of 50 mg. of cortisone and 0.1 Gm. of Dilantin daily.

Case 4. E. N. was a 35-year-old male, gravely ill with Hodgkin's disease. The diagnosis had first been made two years previously. On his fourth admission to the hospital, cortisone therapy was initiated after a thorough laboratory work-up. He received 100 mg. per day, which produced rapid symptomatic improvement; this was supplemented by nitrogen-mustard therapy with good effect.

Six weeks after the beginning of cortisone therapy the patient suddenly collapsed and had a convulsive episode followed by a rise in temperature, weakness, and malaise. Cortisone was temporarily discontinued, and phenobarbital was given. However, since the patient's condition was rapidly deteriorating, cortisone therapy was resumed for one more month; this time there was no symptomatic improvement. Four days after cortisone had been discontinued again, the patient died suddenly. A postmortem examination showed Hodgkin's disease with involvement of the retroperitoneal nodes, right adrenal, both lungs, stomach and liver; thrombosis of the left renal veins; and thrombosis of the inferior vena cava with nodular perivascular involvement. There was also a bilateral obliterating pleurisy.

DISCUSSION

In assessing the circumstances in which clinical seizures occurred in these 4 cases, it should be noted that each patient was chronically and gravely ill. All had an elevated blood sedimentation rate but no disturbance in electrolyte metabolism prior to institution of hormone therapy. Three of the 4 patients suffered from collagen diseases. It is of interest that most of the patients in this series of 43, who suffered from collagen disease had more or less grossly abnormal EEGs prior to hormone therapy, manifested by slowing of activity and mildly paroxysmal features (5).

Dorfman and his collaborators (6), in the course of their investigation on the effects of ACTH on connective tissue, observed status epilepticus in 3 instances. Their patients suffered from rheumatic fever, periarteritis nodosa, and dermatomyositis, respectively. Soffer *et al.* (7) in their series of 17 patients with lupus erythematosus observed 4 in whom convulsions developed early during treatment with cortisone and ACTH; the rest remained seizure-free during long-term treatment with these drugs. In our series, clinical seizures developed in both patients suffering from systemic lupus erythematosus; and status epilepticus occurred in one of them after she had been on the drugs for about fourteen months. In this respect our observations differ from those by Soffer *et al.*

Lupus erythematosus may produce central nervous system involvement, but of our 2 patients with lupus erythematosus who had seizures, one showed no evidence of cerebral abnormalities in the postmortem examination, and the other gave no clinical evidence of central nervous system involvement before the occurrence of status epilepticus.

Another significant observation made on 2 of our 4 patients in whom seizures and psychotic features developed, was a potassium depletion con-

comitant with these complications. McQuarrie (9) and Ransohoff *et al.* (10), among others, have likewise stressed the effects of steroid hormones (ACTH and cortisone) on the potassium and sodium electrolyte balance. They observed that changes in potassium and sodium levels may have a direct bearing on 1) the cortical electrical activity as shown in the EEG, 2) the seizure threshold, and 3) the psychotic and delirious manifestations. McQuarrie's suggestion that "factors which tend to make the internal environment hypotonic also tend to enhance convulsive susceptibility, while factors predisposing to hypertonicity reverse this tendency," is supported by Mote's (11) report of a decrease in the incidence of psychosis in cases in which ACTH dosage was reduced, potassium salts were added, and sodium intake was restricted.

Ransohoff and his collaborators reported dramatic improvement following oral and intravenous administration of potassium in a case of toxic psychosis in which, during ACTH threapy, there were changes in the electrocardiogram, in the level of serum potassium and in the EEG, which were indicative of potassium depletion.

Despite these observations, which may to some degree apply also in some of our cases, we concur with Glaser's (12) suggestion that "the effects of potassium depletion on the central nervous system activity indicate only one phase of more complex processes which take place when complications such as EEG changes, convulsions and psychotic manifestations occur." We consider that the overt symptomatology of a psychosis may, to a certain extent, be determined by the patient's basic personality structure, as well as by his reaction to metabolic and emotional stresses accompanying his illness and the changes produced by therapy.

In agreement with other reports, our 4 patients who had a major psychosis with or without convulsions did not necessarily show evidence of disturbances in the electrolyte metabolism (5). Occasionally, normal brainwave patterns are recorded during an episode of acute and often severe paranoid and schizophreniform behavior [(5) Table 1]. It is hoped that further systematic investigations into the effect of changes in electrolyte metabolism on the activity of the cortex, through electroencephalographic studies combined with thorough psychiatric and psychologic studies, may shed further light on these problems.

SUMMARY

In the course of systematic investigation of the effects of cortisone and adrenocorticotropin in 43 nonepileptic patients, convulsive seizures were observed in 4 instances. Summaries of the 4 case histories and illustrative electroencephalograms are presented. A review of the occurrence of convulsions complicating ACTH and cortisone treatment is given.

Disturbances in the electrolyte metabolism, instability of electrical cortical activity prior to hormone therapy, the influence of the basic personality structure, and emotional stresses accompanying the course of an illness are possible etiologic factors in these complications.

Acknowledgments

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PITUITARY INSUFFICIENCY ASSOCIATED WITH DIABETES MELLITUS

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PITUITARY insufficiency in a patient with diabetes mellitus is sufficiently uncommon to warrant the report of a case including clinical and biochemical investigations, with special reference to the adrenal cortex, thyroid gland and the disordered metabolism of diabetes mellitus.

Simmonds in 1914 (1) described a condition of severe cachexia which might result from a lesion of the anterior hypophysis. Sheehan in 1939 (2) showed that milder cases occur, in which some of the classic symptoms are absent. It would seem, therefore, that not all the anterior pituitary hormones are equally affected, and consequently there are varied clinical and biochemical presentations, such as "pituitary myxedema," "pituitary adrenal failure," and "pituitary hypogonadism." Sheehan and Summers in 1949 (3) stated that the degree of destruction of the gland can be correlated with the severity of the clinical state. Reduction of pituitary function also affects carbohydrate metabolism, as manifested by increased insulin sensitivity and fasting hypoglycemia. In 1942 Houssay and his associates (4) showed the ameliorating effect of hypophysectomy on experimental diabetes. In man, the equivalent of this phenomenon is rare. Only 11 cases have been reported, in which diabetes mellitus has been mitigated by the subsequent development of pituitary insufficiency (5–15). In several of these cases necrosis of the pituitary gland was found at autopsy. One patient died in hypoglycemic coma. In 4 patients the condition developed following a pregnancy.

The case described here presented as one of myxedema, but an intolerance to thyroid therapy and an extreme insulin sensitivity suggested the diagnosis of pituitary insufficiency.

CASE REPORT

A married woman, aged 50 years, gave a history of the onset of diabetes mellitus in 1923. The disease was well controlled by diet and two injections of soluble insulin, 20 units twice daily. She had had two pregnancies, but both babies died soon after birth.

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After the last (apparently normal) pregnancy in 1937, the patient failed to lactate and menstruation became scanty and irregular and stopped altogether soon after, without menopausal symptoms. She then complained of weakness, listlessness, depression, and dread of cold; she was also slow and irritable. The hair on her head and pubis began to thin and axillary hair disappeared. Her cheeks and eyelids became puffy and she gained weight. Eventually the condition was diagnosed as myxedema and she was given thyroid.

In 1940 she attended this hospital for the first time because of frequent hypoglycemia. She was taking a mixed dose of 16 units of soluble insulin and 16 units of protamine zinc insulin daily. On her second attendance in 1952, she was still subject to insulin reactions in the early morning, in spite of a great reduction in the dose of insulin, to 8 units of soluble and 4 units of protamine zinc insulin daily. She was unable to tolerate even 1 grain of thyroid daily, on account of the diarrhea and loss of weight which this small dose invariably precipitated. She was, therefore, admitted for investigation. Skin and hair changes were noted as before and no thyroid tissue could be felt. Breasts were atrophic with little glandular tissue or pigmentation around the nipples. Her external genitalia were also atrophied. Examination of the chest, abdomen and nervous system revealed nothing abnormal. Her blood pressure was 130/80, with no evidence of arteriosclerosis. The ocular fundi were normal, even after diabetes mellitus for thirty years. She had never shown ketone bodies in her urine.

In view of the extreme insulin sensitivity, intolerance to thyroid therapy, clinical findings and the suggestive history, a provisional diagnosis of pituitary insufficiency was made.

Investigations

The results of routine tests (Table 1) were all within normal limits. X-ray examination showed the skull and chest to be normal. The electrocardiogram showed QRS complexes of low voltage in all leads, and the T waves were isoelectric, as seen in hypothyroidism. Eosinopenia occurred following administration of adrenaline (16), as well as of ACTH (17).

Thyroid function

In 1952, the patient was taking 1 grain of thyroid daily, the blood cholesterol level was 300 mg. per 100 ml., and the B.M.R. was −19 per cent. In 1953, after thyroid therapy had been discontinued for three months, the blood cholesterol level was 692 mg. per 100 ml. and the B.M.R. was −38 per cent. The patient was then given 10 microcuries of radioactive iodine (I^{131}). In following the urinary excretion of I^{131} (18), it was found that 76.4 per cent of the dose was excreted during the first forty-eight hours. This indicated failure of uptake of I^{131} by the thyroid gland and was compatible with the diagnosis of myxedema. The patient was then given 12.5 mg. of pituitary thyrotropic hormone[1] (TSH) twice daily for three days, together with 100 microcuries of I^{131}, both before TSH and immediately following the last dose. The uptake was measured over the thyroid, the thigh and the liver. Before TSH, the uptake of I^{131} by the thyroid gland after forty-eight hours was approximately 0.6 per cent, and the neck/thigh ratio, 1.14. The results obtained after TSH approximated those before TSH and may not, therefore, indicate a

[1] The thyrotropic hormone (TSH) preparation was made available through the courtesy of the International Department of Armour & Co., Chicago. Each vial contained 10 U.S.P. units (lot R 527024), equivalent to 25 mg. of thyrotropin in terms of the old Armour House standard 2R3.

TABLE 1. CLINICAL, PATHOLOGIC AND BIOCHEMICAL INVESTIGATIONS

Urine	No abnormalities detected
Erythrocyte sedimentation rate	9 mm., 1st hour (Westergren)
Hemoglobin: Per cent	90 92 94 96
Gm./100 ml.	13.3 13.6 13.9 14.2
White blood cell count	6,100 Neutrophils 36%
	Lymphocytes 54%
	Monocytes 4%
	Eosinophils 6%
Blood film	Normal red cells
	Mild eosinophilia
	Normal platelets
Hematocrit	42 mm./100 mm.
Mean corpuscular hemoglobin concentration	33%
Wassermann reaction	Negative
Histamine test meal	No free hydrochloric acid
Eosinophil response to adrenaline	Decrease from 190 to 80
Eosinophil response to 25 mg. of ACTH	Decrease 240 to 11
Alkali reserve	55 & 56 vols. CO_2 %
Blood urea	26 & 26 mg./100 ml.
Plasma proteins	Total 6.05 Gm. % Albumin 4.16
	Globulin 1.89
	A/G ratio 2.2
Serum sodium	143 mEq./L.
	138 mEq./L.
Serum chloride	93 mEq./L.
	103 mEq./L.
Serum potassium	3.8 mEq./L.
Follicle-stimulating hormone	>24 <48 mouse units

response of the thyroid to the pituitary hormone, although the percentage of iodine taken up by the thyroid was slightly greater (uptake was approximately 1.5 per cent and the neck/thigh ratio was 3.35). There was a loss of body weight, increase in glycosuria and rise of the B.M.R. to +86 per cent (Table 2) following the administration of 75 mg. of TSH, but these may have been nonspecific effects.

Adrenal function

Urinary 17-ketosteroid and adrenal cortisteroid excretion were estimated at frequent intervals and are of interest here, since recent methods in this field do not appear to have been applied to this type of case previously. The total 17-ketosteroid excretion was measured according to the method recommended by the Medical Research Council, Committee on Clinical Endocrinology (19). Fractionation of the 17-ketosteroids was performed by the method of Pond (20). The very low total 17-ketosteroid excretion (1.1 mg. and 0.6 mg. per twenty-four hours *vs.* the normal range for women of 4–14 mg.) represented a failure of one aspect of adrenal function. Adrenocorticosteroids were kindly measured by Miss J. B. Lunnon, using the paper chromatographic method of de Courcy, Bush, Gray and Lunnon (21), and were within normal limits, though the excre-

TABLE 2. SPECIAL INVESTIGATIONS

	On thyroid (1 gr. daily), 1st admission, 1952	Before TSH (off thyroid 3 months), March 1953	After 75 mg. TSH (12.5 mg. twice daily for 3 days)	Before ACTH	After ACTH (25 mg. every 6 hrs. for for 4 days)
Insulin dosage (total)	12 U	8 U	Insulin requirement increased		Insulin requirement increased
Weight (pounds)	120	125	119	119	124
Glycosuria	Minimal	Minimal	Considerable		Considerable
Blood cholesterol (mg./100 ml.)	300	692	600		
B.M.R. (%)	−19	−38	+86		
Urinary 17-ketosteroids (mg./24 hrs.)	1.1	0.6	1.6		5.8; 5.0
Urinary corticosteroids* (μg./24 hrs.)		Comp. E, 30 Comp. F, 70			Comp. E, 100 Comp. F, 800
Total urinary (48-hr.) I^{131} excretion		76.4%	73.1%		
Value of T		1.5	1.5		

* Normal range (paper chromatography)—female: compound E, 30–110 μg./24 hrs.; compound F, 10–120 μg./24 hrs.

tion of compound E tended to be low (Tables 2 and 3). This was consistent with the clinical findings, which were not referable directly to adrenal failure. Following ACTH (25 mg. every six hours for three days), the excretion of corticosteroids and 17-ketosteroids showed a rise similar to that demonstrated in normal subjects in this laboratory. After cortisone therapy, however, there was a depression of the 17-ketosteroids below the initial level, but the rise of corticosteroids was maintained.

The significance of the 17-ketosteroid fractions (labelled I–VIII in Table 3) has been considered elsewhere (22). Table 3 shows the following abnormal features: the lowering of all absolute values of β17-ketosteroids; the relatively raised percentage of the 11-oxy, 17-ketosteroid fractions VI and VII; and the lowering of fractions I, II and III. These fractions are thought to represent probable breakdown products of the corticosteroids, and to give an indication of adrenocortical activity, respectively. Adrenocorticosteroids on the other hand were within normal limits, which suggests that there was a conservation of steroids essential to life. This pattern of steroid excretion may be characteristic of pituitary insufficiency. On the other hand, it may indicate simply a depression of certain adrenal functions, as commonly occurs in other endocrinopathies such as myxedema, in which adrenal atrophy has been demonstrated postmortem (3).

TSH produced only a slight effect on the adrenocortical output. ACTH, however, produced a rise of adrenocortical hormones, and of total 17-ketosteroids (five- to sixfold) commensurate with the maximum rises described after administration of ACTH (23). The fraction which showed the maximum rise was the β fraction—an unusual finding in this laboratory, and not previously reported in this type of patient. The clinical significance of this rise is, as yet, unknown. Variable rises of different fractions have been reported (24–26). The 17-ketosteroid fractionation findings, therefore, suggested alteration of certain aspects of adrenocortical activity, not manifested by direct measurement of adrenocortical steroids in urine. In contrast, after cortisone therapy, there was a depression of all fractions save VI and VII. This probably represented excretion of the cortisone itself, as has been demonstrated in other patients (27). The productions of compounds E and F were greatly stimulated by ACTH and that of E was maintained by cortisone administration.

TABLE 3

A. *17-Ketosteroid Fractionation Studies*

Specimen	Total 17-KS (mg. per 24 hrs.)		Fractions*							
			I	II	III	IV	V	VI	VII	VIII
1	1.1	mg.	0.1	tr	0	0.4	0.3	0.3	0	0
(no treatment)		%	7	3	0	35	25	30	0	0
2	1.6	mg.	0.2	tr	0.1	0.5	0.2	0.4	0.1	tr
(after TSH)		%	10	2	6	31	14	28	6	3
3	5.8	mg.	0.2	0.7	0.7	1.0	1.6		1.5	0.2
(after ACTH)		%	4	11	11	17	28		24	4
4	5.0	mg.	0.5	0.5	0.6	0.6	0.6		2.3	0
(after ACTH)		%	10	0	11	12	12		46	0
5	1.0	mg.	0.1	0.1	0	0.2	0.2	0.4	0.1	0
(after cortisone, 25 mg. daily for 2 wks.)		%	9	5	0	21	23	37	5	0
Normal female (avge. of 9 cases)	8.0	mg.		1.6		2.5	2.9		0.7	
		%		20		31	35		9	

B. *Adrenocortical Steroid Analysis*

Specimen	Δ⁴-3-Ketosteroids				Reducing steroids, glucuronide-conjugated	
	Free			Glucuronide-conjugated		
	Comp. E.	Comp. F.	Comp. X_4†	Comp. X_4†	Tetrahydro-comp. E.	Unidentified compounds
Before treatment	30	70	20	20	60	280
After ACTH	100	800	92	480	400	4,000
After cortisone, 25 mg. daily for 2 wks.	160	120	160	1,600	1,000	1,000
Normal range						
Male	20–200	10–80	20–120	0–200	240–3,000	0–1,500
Female	30–110	10–180	10–320	10–4,000	160–4,000	0–2,200

* Main constituents of 17-ketosteroid fractions:
I, II and III = β fraction
IV = androsterone
V = etiocholanolane
VI and VII = 11-oxy 17-ketosteroids
VIII = unidentified

† X_4 and X_5 are unidentified Δ⁴-3-ketosteroids with R_f values of 0.24 and 0.39 in benzene/50% methanol system.

Studies of the utilization of water as an index of adrenocortical function (28) were not practicable in this patient because of glycosuria.

Follicle-stimulating hormone (FSH)

The result of this estimation was more than 24 and less than 48 mouse units, which is low for a menopausal woman.

Intravenous insulin tolerance test

Increased insulin sensitivity has long been recognized in these patients, and Fraser,

Albright and Smith (29) have suggested that the response of the blood glucose to intravenously injected insulin shows a characteristic curve.

An arrangement of suitable doses of insulin and buffer meals was found, which resulted in a fasting blood glucose level within normal limits. On the day of the test, the blood glucose was followed at first, to ascertain that fasting did not cause a further fall before the patient was given one fifth of the standard dose of insulin (0.1 unit/Kg. of body weight). The rapid drop in the blood glucose indicated increased insulin sensitivity, in view of the small size of the dose given (1 unit of insulin) (Fig. 1). The subsequent failure

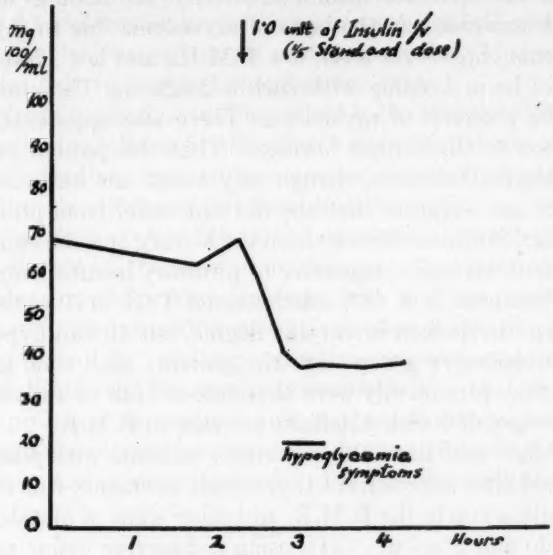

FIG. 1. Insulin sensitivity test.

of the blood glucose level to rise, confirmed the presence of "hypoglycemic unresponsiveness." (11).

Clinical response and progress

During the three days of TSH administration the patient felt subjectively much improved; she lost 6 pounds in weight and showed increased glycosuria, requiring more insulin. Her response to ACTH was similar, though less marked, except for a gain in weight of 5 pounds in four days. It was unfortunately not possible to carry out a "dummy" experiment to assess the psychologic effects of injections; the patient was not a suggestible person.

After the completion of the investigations, the patient was given oral cortisone, 12.5 mg. twice daily, which permitted the dose of thyroid to be increased to 2 grains daily, without untoward side-effects. This observation has also been made at the Mayo Clinic (Sprague, (30)). While taking the two drugs, the patient's general condition improved remarkably, both as regards physical appearance and mental alertness. During cortisone therapy her insulin requirements increased and her diabetes became easier to control,

without hypoglycemia. Ketone bodies were found at times in the urine. Her blood cholesterol level fell progressively from 600 mg. to 204 mg. per 100 ml.

Because of a shortage in the supply of cortisone, testosterone (30 mg. daily) had to be substituted (31). The patient maintained a reasonable degree of subjective improvement, but diarrhea to the extent of four to five motions daily reappeared. This indicated clearly the superiority of cortisone in the treatment of this type of patient.

DISCUSSION

Apart from the increased insulin sensitivity, the findings in the present case could be explained on the basis of myxedema due to thyroid failure. The high serum cholesterol level, low B.M.R., and low 17-ketosteroid excretion would be in keeping with such a diagnosis. The studies with I^{131} confirmed the presence of myxedema. There was apparently some metabolic response to thyrotropic hormone. That this patient excreted some follicle-stimulating hormone, though only about one half the amount expected at her age, suggests that she did not suffer from pituitary insufficiency. But against this evidence stand the history and the clinical findings, which were both strongly suggestive of pituitary insufficiency.

In 1936 Thompson *et al.* (32) administered TSH to 79 patients. Most of them had hypothyriodism in varying degree, but 10 had hypopituitarism. The only unresponsive group were the patients with such marked myxedema that they presumably were thyroidless. Half of the cases of hypopituitarism responded with a definite increase in B.M.R.

In 1936 Bulger and Barr (33) reported 2 patients with pituitary insufficiency, studied after injections of thyrotropic hormone; 1 of these patients responded with a rise in the B.M.R. and other signs of metabolic improvement. Querido and Stanbury (34), using radioactive iodine as an index of thyroid activity found that 5 out of their 6 patients responded to TSH. Presumably the one failure was due to an atrophied unresponsive thyroid gland. Sheehan and Summers (3) in discussing necropsy findings of pituitary insufficiency, found that in a minority of the cases the thyroid was represented by only a small mass of scar tissue. Our patient had suffered from the condition for fifteen years, and although no thyroid gland was palpable clinically, there apparently was a remnant of responsive tissue. Tests for pituitary-adrenal function suggested that ACTH production probably occurred only on a reduced scale. The 17-ketosteroids showed changes after ACTH, in particular, the rise of the two fractions of adrenocortical origin, which indicated that the adrenal cortex was probably not functioning normally previously, even though the excretions of compounds E and F were within normal limits.

The complete absence of ketone bodies in the urine of this patient also raises doubts as to the ability to increase corticosteroids on demand. The

administration of even small amounts of cortisone will produce ketone bodies in this type of patient (15).

Fraser and Smith (11) believe that the intravenous insulin tolerance test is the only one which separates the myxedema of thyroid deficiency from that of pituitary insufficiency. In simple thyroid deficiency associated with diabetes mellitus, sensitivity to insulin is not increased; but there is a delay throughout all phases of the test, as the result of retarded metabolism. The insulin tolerance curve obtained in this patient showed a marked fall to symptomatic hypoglycemic levels, in response to only 1 unit of insulin, with almost complete hypoglycemic unresponsiveness. This type of curve is said to occur only in pituitary insufficiency (11).

Another interesting finding in this patient is the complete absence of any evidence of retinal involvement after thirty years of diabetes mellitus. The disappearance of retinal hemorrhages and exudate following the development of pituitary insufficiency has been described (15).

The history of failure of lactation and cessation of menstruation after a pregnancy, followed by the onset of myxedema a year later are in favor of pituitary damage having occurred in connection with the pregnancy. There remains, however, the possibility that this patient in fact did have thyroid myxedema, and that the pituitary infarction occurred spontaneously at a later date (3). Whichever the case may be, we believe that on the basis of the evidence presented the patient now has partial pituitary insufficiency, which, although not complete, represents a further example of this interesting condition occurring simultaneously with diabetes mellitus.

SUMMARY

1. A case is reported of diabetes mellitus and partial pituitary insufficiency, presenting as myxedema.

2. Thyroid function before and after the administration of thyrotropic hormone (TSH) was tested with radioactive iodine (I^{131}), and on both occasions the thyroidal I^{131} uptake was negligible and suggestive of that observed in myxedema. There was, however, some metabolic response to TSH.

3. Studies of the total 17-ketosteroid, fractionated 17-ketosteroid and corticosteroid excretion before, during and after TSH, ACTH and cortisone administration were carried out. The application of new methods to these investigations has been reported. Corticosteroid excretion was within normal limits, though 17-ketosteroid excretion was minimal. The response of the latter to ACTH (in which three quarters of the 17-ketosteroid was of adrenocortical origin) suggested some previous abnormal function of the adrenal cortex, in spite of normal corticosteroid excretion.

4. The response to ACTH and the extreme sensitivity to insulin supported the diagnosis of pituitary insufficiency.
5. The findings are discussed in relation to similar cases in the literature.

Acknowledgment

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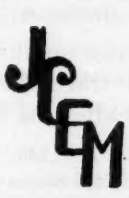

SIMPLIFIED SENSITIVE TEST FOR THYROID FUNCTION, USING PROTEIN-BOUND I^{131}*

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THYROID function has been correlated with I^{131} content of precipitated plasma proteins in experimental animals (1, 2, 3). Several investigators (4–8) have applied this principle as a diagnostic index of human thyroid function and termed the determination "conversion ratio," defined as

$$\text{conversion ratio} = \frac{\text{counts/min. } I^{131} \text{ combined with plasma proteins}}{\text{counts/min. total plasma } I^{131}} \times 100.$$

This ratio, therefore, indicates the percentage of plasma I^{131} in the protein-bound iodine fraction. Excellent correlation has been demonstrated (4–8) between clinical evidence of thyroid function, conversion ratio, and level of protein-bound iodide (PBI) of the plasma. Riggs (9) indicated the difficulties involved in the theoretical interpretation of the conversion ratio but he showed that the method should "distinguish rather sharply between euthyroidism and hyperthyroidism." Albert (19) stated that the major objection to this determination is the large dose of I^{131} required. We have overcome the latter objection by use of an inexpensive scintillation counter.

During the past year the thyroid status of patients has been evaluated by a special radioiodide clinic using as criteria the clinical history, physical examination, BMR, plasma cholesterol level and thyroidal radioactive iodide uptake. Plasma samples from these patients were examined for I^{131} conversion ratio by a separate and independent laboratory. The final diagnosis of the clinic was then compared with that indicated by the conversion ratio data. In final analysis, the conversion ratio correlated with the ultimate clinical impression better than any other single test and was also the simplest determination to perform.

METHODS

The thyroidal radioactive iodide uptake was determined as described previously (11), except that the tracer dose was reduced from 100 to 50 microcuries. Twenty-four hours after the I^{131} was administered, 5–10 ml.

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of venous blood was obtained. A 3–5 ml. sample of plasma was placed in the annulus of the special plastic cup and the radiation was counted on the scintillation counter (Fig. 1). Four or five drops of 3 per cent NaI carrier were delivered into the plasma, and an 8–12 ml. portion of 20 per cent tri-

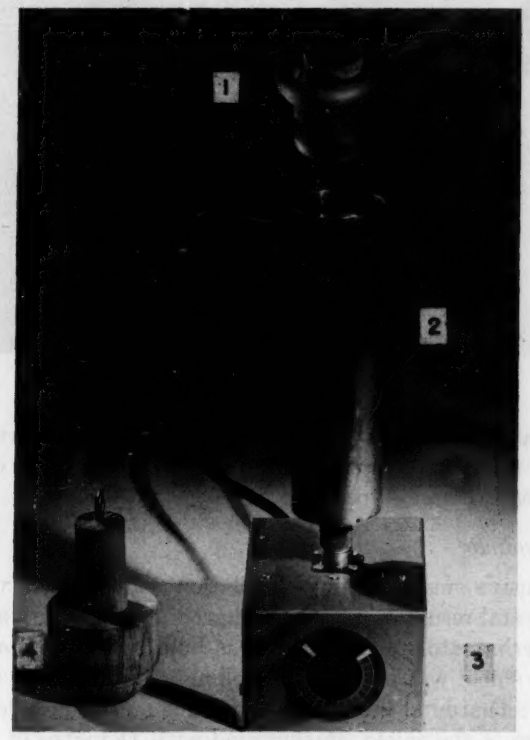

FIG. 1. 1. Plastic cup with 3-mm. annulus which fits around scintillation crystal of unit 2. 3. Especially built discriminator and pre-amplifier. 4. Wooden block which holds annulus cup for centrifuging in 9-cm. centrifuge shield.

choloroacetic acid (TCA) was added to the contents of the plastic cup; then the cup was centrifuged. The supernatant solution was decanted and discarded. The precipitate was washed twice with 10 cc. of 10 per cent trichloroacetic acid. The precipitate remaining in the plastic cup was then counted on the scintillation counter. The conversion ratio was obtained by the expression:

$$\text{conversion ratio} = \frac{\text{count/min. of ppt.}}{\text{count/min. of plasma}} \times 100.$$

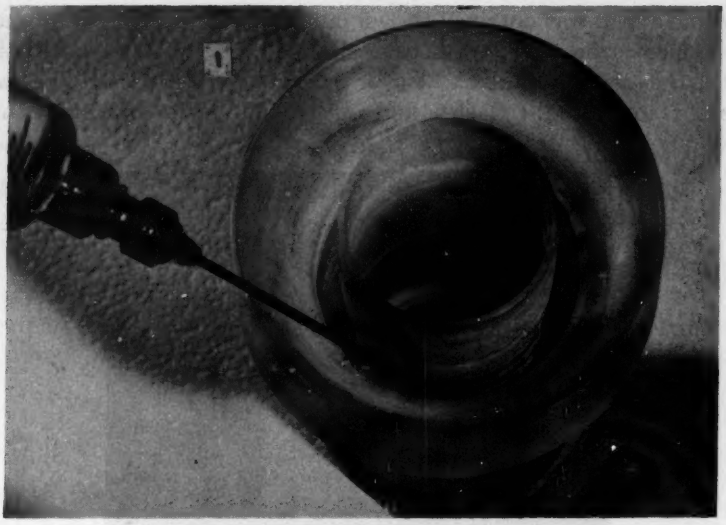

FIG. 2. Detail of plastic cup shown in Figure 1.

Conversion ratios of over 50 per cent are considered indicative of hyperthyroidism. The normal conversion ratio ranged below 50 per cent, and was usually between 10 and 30 per cent.

Scintillation counter

An inexpensive small crystal scintillation counter[1] was used (Fig. 1). The small crystal resulted in a low background count, with an inexpensive shield. A discriminator-preamplifier[2] was built especially for this counter (Fig. 1). The shield was prepared by rolling a sheet of lead ($\frac{1}{16}$ inch × 10 inches × 8 feet) around the scintillation tube and using a lead block as a removable top of the shield. Background with $1\frac{1}{2}$ inches of lead shielding was 68 counts/min. and the plasma counts always ranged from 120 to 1,200 counts/min. above background. It was found that 0.001 μc. of I^{131} counted 280 counts/min. above background, and $3\frac{1}{2}$ inches of lead shielding reduced background to 39 counts/min.

Special annulus cup

A plastic cup (Fig. 2) was constructed to spread the sample over a large surface of the counting crystal and as close as possible to the crystal. This method of counting resulted in very small changes in counts when a sample

[1] Obtained from Nuclear Research Corp., Grays Ferry Ave., Philadelphia, Pa.

[2] Built by B. P. McKay, Department of Physiology, University of Tennessee, Memphis.

was diluted or concentrated. The cup[3] shown in detail in Figure 2 was fashioned from plexiglas on a lathe. The distance between the walls of the annulus was 3 mm. The plastic cup was used as counting cup and centrifuge tube. The TCA was easily added to the plasma in the cup with a syringe, using an 18-gauge needle. The sample and TCA were thoroughly mixed by forcing the mixture in and out of the syringe. This method of mixing was also used to wash the sample in fresh TCA. All volumes were measured

TABLE 1

Patient No.	Conversion ratio %	I^{131} uptake (% of injected dose)
	Thyrotoxicosis	
1[a]	62	46
2	92	58
3	87.8	59
4	94	62
5	61	65
6	74	66
7	94	69
8	81	74
9	72	81
10	93	75
11	58	56
12	98	49
13	89	50
	Nontoxic goiter	
14[b]	5	4.6
15	24	6
16	6.4	6.2
17	14	7
18	17.3	9.2
19	18	10.6
20	8	10.6
21	5	12
22[c]	42	16
23	10	18
24	11.9	30.2
25[d]	5	4

[a] Clinically thyrotoxic.
[b] Taking desiccated thyroid, 2 gr. daily.
[c] Carcinoma of thyroid, with metastasis which accumulated I^{131}.
[d] Myxedema.

[3] Available from Norrell, Inc., 2549 Broad Street, Memphis, Tenn.

with syringes and a volume accuracy of 10 per cent was adequate. Since the ratio was the important measure, even the volume of plasma was not critical. Approximately 2½ volumes of 20 per cent TCA were used for each volume of plasma.

RESULTS

Our results on 25 patients are shown in Table 1. In these patients, the thyroidal radioactive iodide uptake was a better diagnostic test than the BMR or plasma cholesterol level; therefore, the conversion ratio was compared only with radioactive iodide uptake. It will be seen that conversion ratio is as good as, if not more sensitive than, I^{131} uptake as a single laboratory test for hyperthyroidism. The differences between hyperthyroid and normal conversion ratios are usually greater than the differences between I^{131} uptakes in these conditions.

Table 2 shows the conversion ratios of 6 samples of plasma before and after being kept at 50° F. for eight days. The changes due to this storage were of no diagnostic significance.

The method may be of no clinical value if the patient has ingested Lugol's solution or desiccated thyroid within three or four weeks.

We have determined conversion ratios in 2 cases in which the physicians and patients were over two hundred miles from our laboratory. I^{131} (50 microcuries) was sent to the physician, who administered this dose by mouth. Twenty-four hours later he obtained a 5-ml. sample of plasma, which was sent to our laboratory. Conversion ratios were determined satisfactorily on these specimens. This procedure demonstrated that physicians without radiation detection instruments can utilize I^{131} as a diagnostic aid.

SUMMARY

A simplified and inexpensive method for determining conversion ratio

TABLE 2. REPEATED DETERMINATIONS OF CONVERSION RATIO OF PLASMA STORED FOR EIGHT DAYS AT 50° F.

Patient No.	Conversion ratio (%)	
	1st determ.	2nd determ., 8 days later
8	81	81
15	18	13
26*	2.0	3.0
27*	5.3	8.6
28*	13	13.3
29*	66	64

* Thyroidal uptake of I^{131} not determined.

has been described. The method is a very sensitive means for the diagnosis of hyperthyroidism. As outlined here, almost any qualified physician can utilize this diagnostic procedure without investing in special equipment such as radiation detection instruments.

Acknowledgments

We gratefully acknowledge the technical assistance of Miss Jo Turner, M. T. The radioactive iodine was obtained from the Atomic Energy commission.

Addendum

Since this paper was submitted for publication there has been a report from Barrow Hospital, Bristol, England, giving the physical characteristics and excellent theoretical efficiency of a counting method similar to that used in this investigation (Haigh, C. P.: Gamma-ray scintillation counter for weak radioactive solutions, *Nucleonics* **12**: 34, 1954).

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CLINICAL VALUE OF THE PLASMA BUTANOL-EXTRACTABLE (THYROXINE) I^{131} IN THE DIAGNOSIS OF HYPERTHYROIDISM AND MYXEDEMA

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THYROXINE has been accepted generally as the major active hormone of the thyroid gland. It can be separated from nonthyroxine protein-bound iodine and inorganic iodine in plasma by extraction with butyl alcohol (1–4). Radioactive iodine (I^{131}) is incorporated into the thyroxine molecule by the thyroid gland, and the labeled thyroxine can be recovered in the butanol-extractable fraction of the plasma protein-bound iodine (4–6). It is of interest to determine whether or not the rate of formation and release of thyroxine tagged with I^{131} has special value in the diagnosis of hyperthyroidism and myxedema. The present study was undertaken therefore to evaluate the plasma butanol-extractable I^{131} as a clinically useful measure of thyroid function and to compare it with the plasma protein-bound I^{131}.

METHODS AND MATERIALS

Testing procedures

Studies were conducted on 50 euthyroid hospital patients, 12 patients with moderate or severe hyperthyroidism, and 5 patients with primary myxedema. None of the euthyroid subjects had a goiter. The diagnosis in the hyperthyroid and myxedematous patients was unequivocal clinically. The serum chemical protein-bound iodine concentration was determined in each subject and was consistent with the clinical diagnosis. None of the patients had recieved iodine in any form nor treatment with antithyroid drugs. Each subject received 150 microcuries of carrier-free I^{131} orally, after fasting twelve hours. Twenty-four hours later the thyroidal uptake of I^{131} and the basal metabolic rate (BMR) were determined, and a blood sample was withdrawn for determination of the plasma butanol-extractable and protein-bound I^{131}.

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Chemical procedures

For the determination of the butanol-extractable I^{131} (BEI^{131}), a 5-ml. aliquot of plasma was transferred to a 50 ml. glass-stoppered centrifuge tube and extracted twice with 20 ml. of *n*-butyl alcohol. Following each butanol extraction the phases were separated by centrifugation for fifteen minutes at 2,500 r.p.m., and the supernatant liquid (butanol layer) was decanted into a small separatory funnel. The combined butanol fractions were then extracted three times with ½ volume of 4N sodium hydroxide containing 5 per cent sodium carbonate to remove the nonthyroxine I^{131} carried into the butanol phase. The butanol remaining was decanted into a 100-ml. round-bottom flask containing two glass beads, and the separatory funnel was rinsed with 5 ml. of fresh butanol which was then added to the flask. The solution in the flask was concentrated to near dryness by boiling with suction over a water bath. The residue was dissolved in 3 ml. of butanol. which was transferred quantitatively to a flat-bottom counting tube calibrated to 6 ml. This process was repeated with 2 ml. of butanol. The solution in the counting tube then was brought to the 6-ml. mark with butanol. Radioactivity was counted in a well-type scintillation counter. Duplicate determinations were carried out with each plasma sample.

The plasma protein-bound I^{131} (PBI^{131}) was determined as follows. A 5-ml. aliquot of plasma was placed in a flat-bottom 20-ml. counting tube. Plasma proteins were precipitated with 10 ml. of 20 per cent trichloroacetic acid. After centrifugation the supernatant liquid was discarded, and the proteins were washed four times with 10-ml. aliquots of 20 per cent trichloroacetic acid. Care was taken not to pour off or remove protein particles in the process of precipitating, stirring and washing. The solidly packed protein then was counted in a well-type scintillation counter.

Counting procedures

Measurements of thyroidal I^{131} uptake and plasma I^{131} were made with gamma-ray scintillation counters constructed in this laboratory. Sodium iodide (thallium activated) crystals coupled to RCA 5819 photomultiplier tubes were utilized in both counters. For thyroid measurements the crystal and photomultiplier tube assembly was housed in a ¾-inch thick lead cylinder to obtain directionality. To decrease the sensitivity of the counter when using 150-μc. tracer doses, the open end of the counter was shielded with 7.5 mm. of lead, which was more than adequate to absorb backscatter radiation. The effective efficiency of the thyroid gamma counter was 0.005 per cent with the absorber and distance conditions used, and a 150-μc. dose gave approximately 16,000 counts per minute.

A well-type sodium iodide crystal was used for plasma I^{131} counting. The crystal well was ⅝ inch in diameter and 1½ inches deep. The counter was mounted in a specially designed shield made of interlocking lead rings which provided 1½ inches of lead in all directions. The effective efficiency of the well-type gamma-ray counter was 38 ± 1.3 per cent, and 1 μc. of I^{131} yielded a counting rate of 850,000 counts per minute. The background counting rate was approximately 400 counts per minute for both the thyroid and plasma counters.

For determination of the thyroidal uptake, a tracer dose was added to a paper cup containing 100 ml. of tap water, and counted at a distance of 40 cm. from the thyroid counter. The patient drank the water and the empty cup was counted as described. The net tracer dose administered to the patient is then known in counts per minute. Twenty-four hours later a count was made with the tube centered over the thyroid gland at a distance of 40 cm. A lead shield, 1 inch thick and completely covering the gland, was posi-

tioned in front of the neck and the count repeated. The net thyroid count when compared with the net dose count, allowing for decay, gives the percentage uptake by the gland.

A conversion factor between the thyroid and plasma counters made it unnecessary to count the doses in both counters. The thyroid gland and the plasma samples generally were counted long enough to yield a statistical accuracy of 1 per cent or better. However, with low activity, larger counting errors were unavoidable. The BEI^{131} and the PBI^{131} were expressed as a percentage of the tracer dose per liter of plasma. For convenience these values were multiplied by 1,000.

RESULTS AND INTERPRETATIONS

The average values and ranges of variation for the BEI^{131}, PBI^{131}, their ratio, and the ratio of BEI^{131} to total plasma I^{131} are shown in Table 1. The

TABLE 1. PLASMA BUTANOL-EXTRACTABLE AND PROTEIN-BOUND I^{131} VALUES OBTAINED IN 50 EUTHYROID, 12 HYPERTHYROID AND 5 HYPOTHYROID SUBJECTS

	Euthyroid			Hyperthyroid		Hypothyroid	
	Mean	SD	Range	Mean	Range	Mean	Range
BEI^{131}, $(\%/L)\times10^3$	16	9	1–38	273	53–1060	27	9–68
PBI^{131}, $(\%/L)\times10^3$	22	13	5–55	351	59–1440	33	17–75
$\frac{BEI^{131}}{PBI^{131}}\times100$	78	25	24–100	80	68–91	76	48–100
$\frac{BEI^{131}}{\text{Total } I^{131}}\times100$	3	2	0.4–11	53	21–72	1	0.5–3

euthyroid and hyperthyroid groups are completely separated by both the BEI^{131} and PBI^{131}; however, the data obtained in the few myxedematous patients scatter over and beyond the euthyroid range. The ratio of BEI^{131} to PBI^{131} has no apparent discriminatory value, and overlapping of the three groups is extensive. In contrast, the ratio of BEI^{131} to total plasma I^{131}, a thyroxine I^{131} conversion ratio analogous to the protein-bound I^{131} conversion ratio, separates the hyperthyroid and euthryoid groups completely. The hypothyroid patients have low values which overlap into the euthyroid range.

A close relationship between the BEI^{131} and PBI^{131}, particularly in the hyperthyroid patients, is apparent in Figure 1. The correlation coefficient in the hyperthyroid range is 0.997. The average ratio of BEI^{131} to PBI^{131} is approximately four-fifths in both the euthyroid and hyperthyroid groups. Variation about this mean is slight in hyperthyroid patients but marked in the normal subjects. The latter finding and the increasing scatter of

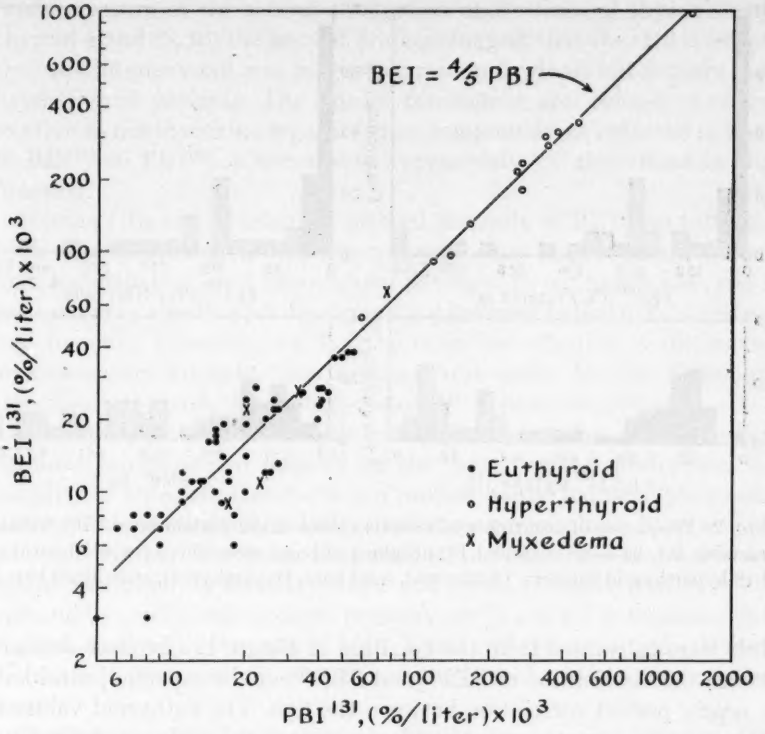

FIG. 1. Relationship between butanol-extractable and protein-bound I^{131}.

points in Figure 1 as the activity values become smaller are most likely attributable to errors of counting, which become relatively prominent as the concentration of activity becomes small.

Figure 2 presents the frequencies of occurrence of the various values of the BEI^{131}, the PBI^{131} and, for comparison, the thyroidal uptake and the BMR in the euthyroid and hyperthyroid patients. In order to compare the graphs, the class interval for grouping the data of each test has been taken as one twentieth of the range of values observed among all cases, euthyroid and hyperthyroid, out to the ninety-seventh percentile point. One hundred per cent of the range was not used for determining scale units, in order to avoid distorting the frequency distributions by occasional extreme values occurring among abnormal patients. In the actual plot of the frequency histograms no case was omitted. With the exception of the BEI^{131}, data were available from 50 euthyroid and 19 hyperthyroid subjects. BEI^{131} measurements were unavailable in 7 hyperthyroid patients, and for these individuals

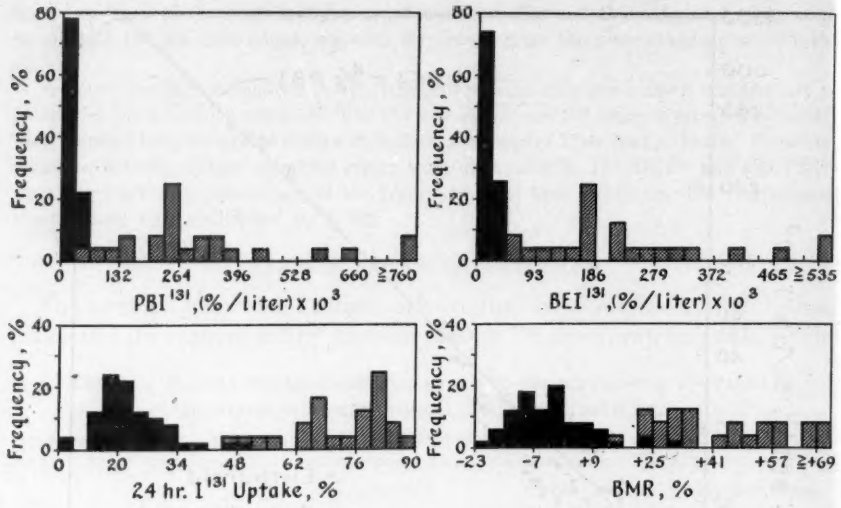

FIG. 2. Frequency of occurrence of various values of the protein-bound I^{131}, butanol-extractable I^{131}, 24-hour thyroidal I^{131} uptake and basal metabolic rate in 50 euthyroid and 19 hyperthyroid subjects. (Euthyroid, solid bars. Hyperthyroid, cross-lined bars.)

values were calculated from the equation of Figure 1. The close similarity between the distribution of BEI^{131} and PBI^{131} is to be expected, considering the nearly perfect correlation between the two. The euthyroid values fall within a very narrow range at the lower ends of the scales, and the hyperthyroid values occupy a very large proportion of the possible range. In contrast the data for thyroidal uptake and BMR show euthyroid values covering approximately half the possible range, and overlapping with the small group of hyperthyroid patients is evident.

COMMENT

A close correlation between plasma levels of nonradioactive butanol-extractable iodine and protein-bound iodine has been observed in both experimental animals and man (1–3). BEI^{131} and PBI^{131} have also been studied (4–10), but their relative clinical usefulness has not been established, other than as a conversion ratio. A high correlation between BEI^{131} and PBI^{131} in the plasma twenty-four hours after administration of a tracer dose, particularly in hyperthyroid patients, was apparent in the present study. These radioactive measures were equally effective in separating the euthyroid and hyperthyroid groups, but neither distinguished between the euthyroid and myxedema groups, and the ratio of BEI^{131} to PBI^{131} was not significantly different among the groups. If one utilizes the BEI^{131} as a

crude measure of the rate of production and release of thyroxine by the thyroid gland (8, 9), the present findings suggest that the rate is essentially the same in euthyroid and in hypothyroid individuals but is more rapid in hyperthyroid patients. The rate of production and release of thyroxine relative to nonthyroxine organic iodine compounds, as reflected in the ratio of BEI^{131} to PBI^{131}, is not affected apparently by alterations in thyroid function.

Harsha (10) and Morton (9) utilized the ratio of BEI^{131} to total plasma I^{131} as a measure of thyroid function and obtained good separation of euthyroid, hyperthyroid, and hypothyroid patients. In our hands also, this ratio has been very effective for discriminating between hyperthyroid and euthyroid patients. However, we find the ratio less effective in distinguishing myxedematous subjects. This observation is similar to that of Sheline and associates (11) with the protein-bound I^{131} conversion ratio.

In general, these tracer techniques reflect rates rather than quantities of hormone produced and released by the thyroid gland. Differences in the quantity of butanol-extractable and protein-bound iodine in the plasma of euthyroid, hyperthyroid and myxedematous persons have been well established (3, 12, 13). The ratio of nonradioactive thyroxine to protein-bound iodine was found by Bassett, Coons and Salter (1) to be similar in euthyroid and hyperthyroid subjects, but very low in one myxedematous patient studied. Analysis of the data of Man, Kydd and Peters (3) similarly reveals this ratio to be essentially the same in euthyroid and hyperthyroid individuals.

Comparisons of the frequency distribution graphs for the BEI^{131}, PBI^{131}, thyroidal uptake, and BMR indicate that the first two measures separate the euthyroid and hyperthyroid groups more effectively. Euthyroid values are confined to a relatively narrow range, hyperthyroid values are spread out, and there is less overlapping of the two groups. The frequency distributions of the PBI^{131} are similar to those reported by Silver and co-workers (14).

The hyperthyroid and myxedema groups of the present report are small, comprising the earliest cases tested in a larger study evaluating multiple measures of thyroid function. However, there appears to be little clinical application for the BEI^{131}, in view of its close correlation with the PBI^{131} and its greater technical difficulty.

SUMMARY

1. The butanol-extractable (thyroxine) I^{131} and the protein-bound I^{131} levels in plasma twenty-four hours after administration of a tracer dose of I^{131} were studed in 50 euthyroid, 12 hyperthyroid and 5 myxedematous subjects.

2. These two measures are closely correlated, the former comprising approximately four fifths of the latter.

3. They separate euthyroid from hyperthyroid individuals very effectively but are not of value in distinguishing myxedematous patients. The ratio of butanol-extractable to protein-bound I^{131} has no diagnostic value. The ratio of butanol-extractable to total plasma I^{131} is similar in discriminative effectiveness to the protein-bound I^{131} conversion ratio.

Acknowledgments

We acknowledge gratefully the competent and conscientious technical help of Miss Maud Dahle and Mrs. Mildred Mixa.

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PSEUDOHYPOPARATHYROIDISM: A CASE REPORT

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THE first 3 cases of pseudohypoparathyroidism were reported by Albright (1) in 1942, and since that time approximately 23 cases have been recorded (2–12). Thus it is still a relatively new and uncommon condition.

The syndrome presents the same clinical features as idiopathic or postoperative hypoparathyroidism, but additional findings are (a) lack of response to parathyroid hormone, (b) a short, stocky habitus with rounded facies, (c) calcinosis cutis, and (d) early closure of some of the metacarpals and metatarsals (2).

Albright thought the condition represented failure of end-organ response as exemplified in the "Seabright-bantam syndrome," in which the Seabright bantam, with a normal level of the male hormone, fails to produce normal male plumage. In pseudohypoparathyroidism the body fails to respond even to excessive amounts of the parathyroid hormone. In fact the lack of phosphorus diuresis after administration of parathyroid extract is given as one of the diagnostic tests (6).

The following case is of interest, in view of the few reports in the medical literature to date.

CASE REPORT

(Santa Clara County Hospital, #39579) A 28-year-old white female was admitted to the hospital on April 9, 1953, because of a dermatologic disorder. It was noticed on perusal of previous records that the case had been diagnosed as hypocalcemic tetany with congenital cataracts. When she was 12 years old, a private physician had been called to the patient's home because of a "fit." When the doctor arrived the child was awake and somewhat lethargic. She had a small cut on her tongue and blood was present on the pillow. Descriptions of the seizures were inadequate until her admission to the Santa Clara County Hospital in 1941, when the following was recorded in the nurses' notes: "Patient screamed. Head turned to side—frothy saliva from mouth. Arms out straight with thumbs into palms, clenched by fingers."

Laboratory studies at the time of her first admission in 1941 showed a marked hypocalcemia and hyperphosphatemia. She was subsequently given oral calcium salts and daily injections of from 25 to 125 units of parathyroid extract. Calcium medication and parathyroid extract were continued for a brief period after the patient was discharged. Despite this regimen, the seizures continued. She then went under private care until the

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* Intern, 1952–1953.

1953 admission, when she stated she had been without seizures for one and a half years while taking diphenylhydantoin sodium, 0.3 Gm. daily, and an undetermined amount of phenobarbital.

At the age of 13 the mother placed her under the care of an ophthalmologist who diagnosed congenital cataracts. Since that time she had undergone approximately four "needling" procedures for this condition.

Past history and family history revealed a maternal uncle with idiopathic grand mal seizures. All other members of the family were without any signs or symptoms of the patient's disorder (5). The patient had had one brief episode of infantile diarrhea, measles, pneumonia, and occasional "colds." The only surgery had been for cataracts and for removal of a needle fragment in the soft tissues of the back; the needle had broken when the patient had a seizure while pneumoencephalography was being attempted. All but six of her teeth had been extracted because of cavities. There was no previous history of thyroid, renal or pancreatic disease. She completed high school with average grades, retarded only by hospital confinements for diagnosis and surgery.

Physical examination revealed a short, markedly obese, white female (Fig. 1) with pronounced roundness of her face, and glasses with extremely high powers of magnification. Her height was 61 inches and weight 146 pounds. Her extreme placidity bordered

FIGURE 1

on lethargy. She had residual pupillary deformities from previous surgery and wore partial dentures. There was slight shortening of the fifth fingers bilaterally, but no absence of knuckle prominences. Bilateral woody-type edema was noted in the pretibial areas. There was an extensive crusted, weeping, erythematous, and purulent involvement of the skin of the anterior abdominal wall, upper aspect of the thighs, and labia. Trousseau's and Chvostek's signs were marked, and superficial and deep reflexes were diminished or absent; this was substantiated after the initial examination by four different observers.

Serum calcium and phosphorus levels on the day following admission were 5.6 and 7.7 mg. per 100 ml., respectively. Results of serologic tests were negative and a complete blood count and urine analysis gave findings within normal limits, as did a glucose tolerance test (8) and analyses of the blood for urea nitrogen, fasting sugar, alkaline phosphatase, protein-bound iodine, total proteins and A/G ratio. Urinary 17-ketosteroids were normal. Following the intravenous administration of 200 units of parathyroid extract, the patient's urine showed a phosphorus diuresis of less than 10 milligrams. X-ray examination of the skull showed bilateral calcification of the basal ganglia (Fig. 2) and hypoplastic, malposed and malformed teeth. An asymmetry of the metacarpal bones was noted, with the second metacarpal being inordinately long and the fifth metacarpal shortened (Fig. 3). All visualized bones were demineralized.

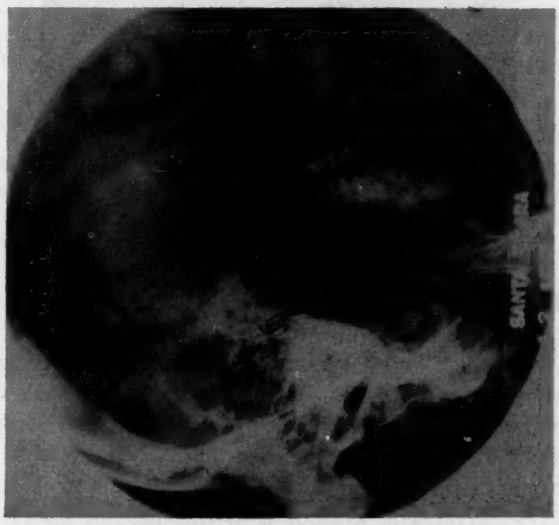

FIGURE 2

After the initial diagnostic studies were performed, the patient received dihydrotachysterol capsules in a total daily dosage of 8 milligrams, oral calcium salts, and aluminum hydroxide gel. Several typical tetanic seizures were observed by the house staff during this admission; these were terminated by the intravenous administration of a 10 per cent solution of calcium gluconate. The dihydrotachysterol was gradually increased to 15 milligrams per day and vitamin D was added in a dosage of 200,000 units daily. On this regimen the patient's blood calcium and phosphorus levels and urinary calcium (Sulkowitch test,) reverted to normal limits within a period of approximately six weeks. The concentration of serum calcium on May 29 was 9.3 mg. and of phosphorus, 4.9 mg. per 100 ml. Reduction in the dosage of dihydrotachysterol was then begun, in order to establish a maintenance level. Her lethargy improved to the point where she was considerably more alert and did not object to increasing physical activity.

DISCUSSION

Because of hypocalcemia, hyperphosphatemia, tetany, congenital cata-

FIGURE 3

racts, the characteristic physical habitus and facies, dyschondroplasia of metacarpals, diminished response to intravenous parathyroid extract, the presence of Chvostek's and Trousseau's signs, and basal ganglia calcification, this patient represents a case (previously undiagnosed) of pseudohypoparathyroidism, or the so-called Seabright-bantam syndrome. Electroencephalography revealed a diffuse abnormality consistent with a convulsive order. Some of the congenital anomalies often seen in this condition may sometimes be present without a disturbance of calcium and phosphorus metabolism (13). Elrick *et al.*, in their report of 4 new cases, give graphic evidence that a patient with the Seabright-bantam syndrome may show an increased urinary phosphorus excretion of from 5 to 50 milligrams following intravenous administration of parathyroid extract (14). Albright confirms the writer's opinion that this patient's urinary phosphorus diuresis was not inconsistent with the diagnosis (15).

SUMMARY

A case of pseudohypoparathyroidism in a 28-year-old female is reported. There was no familial evidence of the disease. She responded to administration of dihydrotachysterol, oral calcium salts, aluminum hydroxide gel, and vitamin D with a reversal of serum calcium and phosphorus levels to normal limits and a cessation of tetanic seizures. Previously reported cases are cited and the characteristic manifestations of the syndrome noted.

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Teaching Clinic

ALKALOSIS, RENAL INSUFFICIENCY AND HYPERCALCEMIA SECONDARY TO THE EXCESSIVE INTAKE OF SIPPY POWDERS

FOR many years it has been recognized that alkalosis can be a complication of the Sippy treatment of active peptic ulcer. In a few reported cases, the alkalosis has been complicated by associated hypercalcemia and renal insufficiency. Our case is presented because it illustrates problems which confront the internist in the diagnosis of this clinical syndrome.

REPORT OF CASE

A 49-year-old farmer, was admitted to the clinic in August 1953, in a critical condition, with almost constant nausea and vomiting. His family reported that he had been hospitalized at home for several weeks because of nausea and vomiting, without melena or hematemesis.

He was dehydrated; his blood pressure was 80 mm. of mercury, systolic, and 60 mm., diastolic. The heart and lungs were normal, except for sinus tachycardia. The pulse rate was 132. Examination of the ocular fundi showed only minimal narrowing of the retinal arterioles. The vomitus was dark brown but did not contain blood. Results of physical examination otherwise were not remarkable.

The pertinent laboratory data on the day of admission and thereafter are outlined in the table. The specific gravity of the urine was 1.013; the reaction was acid, and albuminuria of grade 2 (on a basis of 1 to 4) was present. Pyuria was graded 3, and a few erythrocytes were found.

Initial treatment included intravenous infusions of isotonic solution of sodium chloride and 5 per cent glucose. To each liter 40 mEq. of potassium chloride was added. Twenty-four hours after the patient's admission, vomiting had stopped and the patient was much improved; the laboratory findings were as depicted in the table. In view of the totally unexpected hypercalcemia, the diagnosis of hyperparathyroidism with secondary renal insufficiency or vitamin D intoxication was considered. Roentgenograms showed the teeth and hands to be entirely normal.

By the third day the patient had improved sufficiently to be able to give a reliable history. He said he had not had any vitamin D. For about twenty-five years he had noted recurrent bouts of burning epigastric distress occurring thirty to sixty minutes after meals, occasionally between meals, and rarely at night. Milk did not relieve the distress and, in fact, caused "sour stomach." Twenty years ago his physician had prescribed Sippy powders, composed of calcium carbonate and sodium bicarbonate. He had become rather dependent on these for relief, and estimated his average intake of powders to be 2 to 2½ pounds per week. Because of his distaste for milk, he seldom drank more

TABLE 1. PERTINENT LABORATORY DATA IN ALKALOSIS, RENAL INSUFFICIENCY AND HYPERCALCEMIA FROM SIPPY POWDERS

Constituent or factor	Day of admission	Next day	Sixth day	Eleventh day
Urea, mg.*	234	270	78	58
Creatinine, mg.*	—	14	2.8	2.4
Sulfate, mg. SO_4†	—	44.5	—	4.8
Sodium, mEq./L.	145	148	—	134
Potassium, mEq./L.	2.9	3.3	—	4.3
Chlorides, mEq./L.	61.4	77.7	110	104.5
CO_2, mEq./L.	41.2	39	19.7	17.6
Calcium, mg.†	12.9	—	9.1	9.5
Inorganic phosphate, mg.†	4.8	3.9	—	2.5
Alkaline phosphatase (Bodansky units)	3.2†	—	—	—
Hemoglobin, Gm.*	14.3	—	—	—
Serum proteins, Gm.†	Alb. 5.2 Glob. 2.9	—	—	—
Bromsulphalein retention in 1 hour	20%	—	—	0%
Serum bilirubin	Indirect, 0.9 mg.† Direct, 0 mg.	—	—	—

* Per 100 cc. of blood.
† Per 100 cc. of serum.

than one glass daily. In 1938 he had had several bouts of severe renal colic, after which he passed two renal calculi. In 1948 he experienced nausea and vomiting for several weeks, and may have had hematemesis on one occasion. Roentgenograms of the esophagus, stomach and duodenum were reported as showing nothing abnormal at that time. He had consumed approximately a pint of whisky a day for years. In 1950 cholecystectomy for gallstones had been performed. The surgeon could not detect a duodenal ulcer at the time of surgical exploration.

A diagnosis of chronic nephritis had been made in 1951 because of recurrent nausea and vomiting. The patient had been told that his kidneys were badly damaged. The value for blood urea was elevated at that time, but slowly returned to normal after the parenteral use of fluids and relief from vomiting.

In view of the history of a large intake of Sippy powders, it was considered that the so-called milk-alkali syndrome might be present, despite the insignificant intake of milk. A strict ulcer diet was prescribed, with the use of milk substitutes, 2 teaspoonfuls of a compound of magnesium trisilicate and aluminum hydroxide (Gelusil) to be taken thirty minutes before and after meals, and 50 mg. of methantheline bromide (Banthine) to be taken before meals. The use of all absorbable alkalies was withheld. Subsequent studies with the slit lamp showed no deposits of calcium in the conjunctiva or cornea of either eye.

The patient improved rapidly, and within one week was up and about the ward. The calcium content of the blood returned to normal on the fifth hospital day. His blood pressure had increased to 124 mm. of mercury, systolic, and 82 mm., diastolic. A roent-

genogram of the esophagus, stomach and duodenum revealed no evidence of esophageal varices, or gastric or duodenal ulceration. A roentgenogram of the kidneys, ureters and bladder disclosed no abnormality.

On the day before this patient's dismissal, results of all laboratory studies were normal except for slight elevation of the value for blood urea and mild depression of the carbon-dioxide combining power. The urine had a specific gravity of 1.002, but was otherwise normal. The patient was dismissed on the twelfth hospital day, with instructions to follow a liberal ulcer diet. Gelusil also was prescribed. He was asked to return in three months for re-evaluation of his gastrointestinal complaints and studies of his renal function. In a recent communication the patient wrote that he had been in excellent health and had been working full time since his dismissal from the clinic.

COMMENT

Grant (1) in 1922 was the first to report alkalosis as a complication of the Sippy treatment for peptic ulcer. It remained for Hardt and Rivers (2) in 1923 to report the first detailed observations concerning the symptoms of toxicity which may develop in patients who ingest large amounts of absorbable alkali. The significant symptoms noted were a distaste for milk, anorexia, nausea, weakness, vomiting and clouding of the sensorium. In their cases laboratory studies revealed elevation of the value for blood urea, a normal or increased plasma carbon-dioxide combining power and varying amounts of albumin, casts and erythrocytes and leukocytes in the urine. After removal of absorbable alkalies from the diet, there was a rapid clearing of the symptoms, with gradual return of the renal function to normal.

Many subsequent workers (3–5) have corroborated the findings of Hardt and Rivers. The underlying mechanism of renal failure in these patients has not been clarified, despite considerable clinical investigation. Kirsner and Palmer (5) demonstrated that alkalosis may appear in the absence of antecedent renal impairment. In their study several patients with known impairment of renal function were given large amounts of absorbable alkalies without the subsequent development of alkalosis.

However, other investigators (4, 6) believe some antecedent renal damage may exist in patients in whom alkalosis develops while they are receiving alkaline powders. Berger and Binger (6) stressed the importance of studying the renal function of any patient for whom a Sippy regimen is contemplated. They felt that in the presence of mild degrees of renal insufficiency, the threshold of safety is less, and that the renal injury may be increased concomitantly with the alkalosis. Nevertheless, in most cases, alkalosis does not cause a persistent diminution of renal function, although a diminished clearance of urea may be demonstrable for several months after the alkalosis has been corrected.

In 1936, Cope (7) reported 4 cases in which alkalosis with hypercalcemia complicated the treatment of gastric ulcers with alkaline powders. Despite the very high content of calcium in the blood, the value for blood calcium promptly returned to normal in all cases within ten days after removal of

the absorbable alkalies from the diet. Cope's patients had been ingesting large amounts of calcium carbonate.

It remained for Burnett and co-workers (8) to document carefully 6 cases in which the clinical picture of hypercalcemia without hypercalcuria or hypophosphatemia, calcinosis, and renal insufficiency was presented after the prolonged intake of milk and absorbable alkalies. All their patients manifested the conjunctival or corneal lesions, or both, previously described by Walsh and Howard (9) as occurring in patients who had hypercalcemia. The conjunctival lesions are small, glasslike particles in the conjunctiva near the region of the palpebral fissures. The corneal changes are hazy, grayish, granular epithelial and subepithelial deposits running concentrically with the limbus on either nasal or temporal sides or both.

SUMMARY

When first seen, our patient presented a difficult diagnostic problem. Initially, the clinical and laboratory findings suggested chronic, diffuse nephritis with renal insufficiency. The alkalosis with hypochloremia and hypokaliemia could be attributed to protracted vomiting, with a resulting deficit of electrolytes. The absence of hypertension and the presence of normal ocular fundi, a normal hemogram and the rather minimal urinary findings were against, but did not exclude, primary nephritis. Primary hyperparathyroidism with secondary renal damage was seriously considered when the hypercalcemia was reported. When the patient improved clinically, so that the history could be reviewed, it was recognized that his clinical picture might possibly have a basis in the excessive intake of Sippy powders.

The correction of hypochloremia and hypokaliemia by the parenteral administration of fluids led to prompt cessation of vomiting. The rapid clinical improvement in the patient's condition and the return of renal function almost to normal within a 10–day period constituted a dramatic therapeutic response. Hypercalcemia associated with primary hyperparathyroidism is not appreciably altered when the patient subsists on a diet low in calcium.

Physicians who continue to prescribe absorbable alkalies in the management of peptic ulcer must be on the alert constantly for the possible development of this toxic clinical picture, with the associated findings of alkalosis, hypercalcemia and renal insufficiency.

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Editorial

MULTI-COMPARTMENTED BIOLOGIC MODELS

REPRESENTATION of physiologic events by means of diagrams or models has always been a useful mnemonic and valuable teaching aid. One need only recall the artful "teratomas" of Fuller Albright which have been so invaluable to two decades of medical students attempting to grasp the vagaries of calcium metabolism in bone disease. The model as generally applied to metabolizing systems has attempted to portray the zones in the body where the particular metabolite may be found, the alterations and transfers which it undergoes, and the influences which may be brought to bear at various stages in the metabolic processes. The magnitude of the various stages and the rates of transfer from one stage to the next are indicated only approximately at best.

With the development of isotope tracer techniques the biologic model promises to gain a more accurate portrayal of metabolic events, for by suitable employment of labeled metabolites it is possible in certain instances to define anatomically or chemically the various components of the metabolizing system, to measure each component, and to assign rates of transfer between components. Within the limit of the experimental accuracy of the data, a quantitative and dynamic model of the entire metabolic process may be obtained. Few analyses of this type are at present available. The thyroid-iodine system has lent itself particularly well to studies of this sort because of the unique role played by iodine in this process, the availability and suitability of the isotope, and the relative ease of measurement. Oddie (1), in an excellent theoretical treatment, has calculated from available data various parameters of iodine metabolism in different states of thyroid function. Keating *et al.* (2) and others (3, 4, 5, 6) have also subjected iodine metabolism to mathematical analysis. Similarly, body fluids and electrolytes have been studied by Cardozo and Edelman (7) and by Levenson *et al.* (8), and the ventilatory apparatus by Fowler *et al.* (9).

A model of a biologic system is constructed by defining the compartments and the routes of transfer between compartments. Although a compartment in a biologic system is conventionally thought of as a volume limited by real barriers, the concept may be generalized "so that a substance, such as a chemical element, can be considered to be in a different 'compartment' when it is in a different state of chemical combination" (Sheppard, 10). A

compartment, then, is a component of the body, of homogeneous composition, which is delineated physically but not necessarily anatomically and which mixes uniformly with an increment of the same substance which is introduced into the compartment. These are in essence the basic conditions imposed by Solomon (11) for a compartmented model. It is obvious, however, that unless discretion is exercised in the choice of compartments, the number of elements will increase to a point where the problem cannot be attacked by any method of mathematical analysis.

Success in the use of models in biologic problems lies in the choice of the minimum number of compartments which retain the elements of the problem under consideration. Fortunately, the number of compartments may often be reduced without doing serious violence to facts. Thus, if interactions between certain compartments occur rapidly, the compartments may often be advantageously lumped into a single one over the period of interest. For example, mixing of a substance introduced into the plasma with one or more diffusion spaces usually occurs in a matter of minutes and may be virtually complete before observations on a phenomenon covering a time scale of hours or days are commenced. In this case, it would be valid to consider the plasma and diffusion spaces as one compartment. On the other hand, a compartment with a very slow rate constant of transfer may often be ignored completely over limited time periods.

In many cases, different chemical forms of an element within one physical location may be lumped into a single compartment. For example, iodine is known to be present in the thyroid gland in a number of chemical combinations. However, for practical purposes, these forms may be considered to constitute a single compartment of iodine. On the other hand, if the study is aimed at determining the formation of various compounds within the thyroid, this simplification would preclude any useful results from model analysis.

The rate constant of transfer from one compartment to another is that fraction of one compartment which enters another in unit time. In the general case, two rate constants are defined between any two compartments, representing transfer of material in two directions. Here again, the success of the model lies in the choice of a minimum number of rate constants. The simplest case would be a single rate constant or "clearance" of one compartment into another. A familiar example is renal clearance. If one knows the clearance rate as familiarly expressed in volume of plasma per unit time, and the quantity of the metabolite freely exchangeable with that of the plasma (the compartment), then one can calculate the fraction of the compartment cleared by the kidney in unit time. This is the rate constant of transfer of the compartment to the outside via the urinary pathway. Normally the rate for iodide clearance is approximately 0.07 per hour,

more often expressed as 7 per cent of the iodide compartment per hour.

The discussion of the model has been limited to stable compounds. Essentially, the model is a representation of the processes which the stable compounds undergo in steady-state conditions. Since labeled compounds are identical chemically with their stable counterparts, the rate constants apply equally well to them. Observations of these labeled substances provide information concerning the model and yield quantitative values for the rate constants and also compartment magnitudes. If a labeled substance leaves a compartment with a single rate of transfer, the amount of the labeled substance in the compartment yields a straight line when plotted semilogarithmically against time. The slope of this line is the rate constant. The simplest mathematical quantity derived from this line is the half-time of transfer, or the time which elapses for the quantity of the labeled metabolite in the compartment to change by one-half. The half-time is related simply to the rate constant. The turnover time, or mean time of transfer, can also be calculated and again is simply related to the half-time and the rate constant (Zilversmit *et al.* (12)). From the intercept of the straight line at zero time, one obtains the magnitude of the compartment.

It is oftentimes difficult or impossible to isolate a given compartment for study. More often the resultant of a series of metabolic processes between multiple compartments must be analyzed. Frequently, observations on the compartment of most direct interest may be impossible, and information must be obtained by observations on another compartment. Thus, the appearance of a labeled metabolite in the urine or feces, or its disappearance from the blood may reflect processes occurring remotely in the body. The observed curve may or may not be a straight line when plotted semilogarithmically. More often than not, the curve is a composite of a series of exponentials representing several metabolic events. Under certain conditions the graphic method of "curve pealing" may yield the individual exponentials and corresponding rate constants. From the extrapolated intercepts, the various compartment sizes may be calculated. One must be wary of assigning physiologic significance to these derived parameters, for the data may be analyzed in more than one way and the experimental error in the data may introduce uncertainty. It is permissible, however, for purposes of hypothesis to assign specific roles to each exponential, and to compare and contrast fluctuations in parameters in various disease states or under varying imposed conditions. Thus, one of the exponentials derived from the appearance of labeled calcium in the urine may or may not represent the compartment of bone recrystallization, but it is of considerable interest to compare the size of this compartment and its rate constant in a patient with thyrotoxicosis before and after treatment. An-

other exponential may possibly represent ion exchange at the surface of bone. Its characteristics in the thyrotoxic patient might profitably be compared to those of a patient with another disease characterized by high cardiac output and increased bony surface, *e.g.*, Paget's disease (13).

The biologic model may be of any order of complexity. It may involve two or more compartments in series, with rate constants connecting each to the next (catanary model). One compartment may be derived from, or feed into several other compartments, with different rate constants for each (mammillary model). The model may be complicated by internal cycling, as in the return of iodide derived from hormone degradation to the thyroid gland. Replenishment from external sources and leaks may further complicate matters.

It is a relatively simple matter to write the differential equations for any particular biologic model, but when the model is more complex than even a three-compartmented closed catanary system the mathematics involved in a complete solution of the equations becomes exceedingly difficult or virtually impossible. Yet solution or its equivalent is necessary if one is to compare the results of experimental obervation with those expected from theory, and thereby test the validity of the model.

A convenient escape from this dilemma is provided by the analogue computor as described by Brownell, Cavicchi and Perry (14). This device consists of an electrical network which simulates the biologic model. Thus capacitance represents a compartment, and charge, the amount of labeled material in the compartment. The transient voltage resulting from the charge introduced at zero time is seen on the face of a cathode ray oscilloscope, and is analogous to the specific activity curve. The analogies which are most useful are shown in Table 1. By suitable adjustments of constants and time scales the analogy is made quantitative. It is thus possible to construct a hypothetical model and introduce into the computor assumed values of the various rate constants and compartment sizes. One can then compare the specific activity curves experimentally observed with those obtained from the computor. If the curves cannot be made to superimpose,

TABLE 1. ANALOGIES FOR THE ANALOGUE COMPUTOR

Biologic term	Unit	Electrical term	Unit
Compartment size	gram	Capacitance	farad
Labeled component	per cent	Capacitor charge	coulomb
Rate constant	per cent/unit time	Resistor-amplifier	ohm
Specific activity	per cent/gram	Voltage	volt

it is safe to assume that the model is wrong. If they do superimpose the model cannot be assumed to be the correct one, but would appear to be a reasonable one with which to proceed. In addition, tentative values of the rate constants and compartment magnitudes are obtained from this trial and error approach.

One example from a study of iodine physiology will be mentioned. In a study of iodine-deficient subjects it was observed that labeled iodine accumulated rapidly in the thyroids of some of the patients and then disappeared slowly in simple exponential fashion (15). Other patients who collected labeled iodine equally rapidly proceeded to lose it from the gland rapidly during the first three or four days before attaining a simple but slower exponential loss. The reason for the differences in the two types of retention curves was not apparent until it was observed that those patients with the early rapid drop in the thyroidal retention curve were precisely those who by calculation had the smallest quantity of stable iodine in their glands. An explanation was sought by recourse to models and analysis with an analogue computor.

Iodine metabolism is most simply conceived of as involving three compartments: 1) the iodide of the blood and extracellular space including that not chemically bound in the thyroid, 2) the chemically bound iodine of the thyroid gland, and 3) the extrathyroidal organically bound iodine. Transfer is considered to occur from the iodide compartment to the thyroid, from the thyroid to the extrathyroidal protein-bound iodine, and thence back to the iodide compartment. Excretion may occur both from the iodide and the extrathyroidal organically bound iodine compartments.

On the basis of the three-compartment model it might be expected that the retention curve of iodine would show an initial rapid phase of disappearance as labeled hormone leaves the gland. When sufficient iodide has become available from peripheral degradation of hormone for reutilization by this gland, there would be stabilization of the decay curve at a slower rate of disappearance. Thus, a biphasic curve might well be missed in a subject with a large store of hormone in the gland, but in the impoverished gland the rate constant of release of hormone might be so large as to reduce significantly and rapidly the observed retention of labeled iodine by the gland before reutilization would become a modulating factor.

It was accordingly of interest to observe the similarity of the experimental *in vivo* retention curves from patients with those obtained from a computor analysis based on the three-compartment model. As expected, the computor curves were identical with the observed retention curves for those patients with large amounts of iodine in their glands. However, when the data from patients with small amounts of iodine in their glands were

introduced into the analogue computor, using the three-compartment model, the computor curve was grossly different from the curve observed *in vivo*.

An alternative hypothesis was that the thyroid gland contributes two compartments to the thyroid-iodine system, each with its own rate of release of labeled iodine. One of these was conceived as a large compartment with a slow release rate and the other as a small compartment with a rapid release constant. When this model was set up on the computor, using data from patients with small amounts of iodine in their glands, the computor curves were found to superimpose satisfactorily upon the observed curves.

The result by no means proves the validity of the four-compartment model of iodine metabolism. However, there is a histologic analogy for the two inferred compartments of the thyroid. One compartment might be the organic iodine of the cells where, under conditions of severe iodine depletion, hormone is made and rapidly secreted directly into the blood; and the other, the colloid where a much larger amount of iodine is stored but from whence mobilization of hormone proceeds more slowly. The severely depleted gland would therefore show the effects of the activity of the first compartment, whereas in the replete gland the colloid compartment would dominate the observations and indeed render the cell compartment statistically negligible.

Analysis of tracer data derived from metabolizing systems is certain to have increasing utility, for not only are the dissection of the body into its compartments and the assignment of rate constants legitimate aims of descriptive biology, but indeed the essence of a disease may be an altered rate constant or a change in the dimensions of a compartment. For example, the essence of thyrotoxicosis (although not its etiology) is an altered release rate of the thyroid hormone. Experiments designed to demonstrate and explore metabolic pathways, precursor-product relationships, rate controls, metabolic errors, and drug effects will especially profit from model analysis. The metabolism of calcium, iron, iodine, and trace elements and those experiments in which labeled compounds are introduced and labeled products measured, seem particularly suitable for this sort of study.

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Letter to the Editor

SODIUM-RETAINING CORTICOID IN THE URINE OF NORMAL CHILDREN AND ADULTS AND OF PATIENTS WITH HYPOADRENALISM OR HYPOPITUITARISM

TO THE EDITOR:

When urine from edematous patients with lipemic nephrosis, cardiac failure, or hepatic cirrhosis is acidified to pH 1.0 and extracted for thirty to forty minutes with chloroform, the extracts show sodium-retaining activity, which is highest in those patients with the greatest impairment of sodium excretion (1, 2, 3, 4).

A corticosteroid which causes marked sodium retention and potassium diuresis in adrenalectomized rats has been separated by chromatography from such extracts (4). This sodium-retaining corticoid resembles aldosterone (electrocortin) in several physical, chemical, and pharmacologic properties (4, 5).

Comparable extracts and fractions of urine from normal children and adults on an unrestricted sodium intake are inert or may increase the excretion of sodium, with the bioassay method used (4). When sodium intake is restricted to 10 mEq. per day, measurable sodium-retaining activity appears in the extracts of normal men (6).

METHOD

Urine was acidified to pH 1.0 and shaken four times with chloroform; large doses of this extract did not cause sodium retention. After standing for twenty-four hours at pH 1.0 and 20° C., the urine was extracted again with chloroform: this extract was used in the present studies. The extract was washed with 0.1 volume of cold N/10 NaOH and dried over sodium sulfate. The chloroform was removed by evaporation at 40° C. under reduced pressure. The residue was chromatographed on filter paper in toluene and propylene glycol (7). Sodium-retaining activity was recovered from the fraction moving near cortisone in this system. In a system of benzene and aqueous methanol (8), the sodium-retaining corticoid moves more slowly than cortisone (4). In these systems, the chromatographic behavior of the sodium-retaining corticoid resembles that of aldosterone.

RESULTS

Results of bioassay of the active chromatographic fraction, obtained from the toluene and propylene-glycol system, are presented in Table 1. The data are expressed as the dose of desoxycorticosterone acetate (DCA) which would produce an equivalent response (9). Since the sodium-retaining corticoid and aldosterone are 20 to 30 times as active as DCA in this bioassay, the actual quantities measured are presumably 3.3 to 5 per cent of the weights given in the table (10). The result of each bioassay is cal-

TABLE 1

Sex	Age (yrs.)	17-KS (mg./day)	Sodium-retaining corticoid	
			K/Na bioassay	Na output bioassay
			μg. DCA/day	μg. DCA/day
		Normal children		
M	4	1.1	50 (±19)*	67 (±34)
M	4	2.2	71 (±19)	85 (±34)
F	7	3.6	44 (±19)	71 (±34)
F	8	—	57 (±30)	30 (±54)
			Avge. 55	63
		Normal adults		
F	23	—	98 (±20)	98 (±36)
F	31	14.5	105 (±20)	113 (±36)
M	29	23.2	81 (±20)	95 (±36)
M	31	20.5	55 (±20)	75 (±36)
M	41	17.0	89 (±20)	110 (±36)
			Avge. 86	98
		Addison's disease		
M	49	5.7	**−13 (±20)	−27 (±36)
		Bilateral adrenalectomy		
M	60	4.6	−5 (±19)	−29 (±34)
M	74	5.4	−6 (±19)	−12 (±34)
		Hypopituitarism		
M	52	3.4	85 (±20)	107 (±36)
M	43	4.9	61 (±20)	55 (±36)

* Figures in parentheses indicate 95% confidence limits of bioassay.

** Minus result indicates effect opposite to that of DCA, *i.e.*, increased sodium output.

culated on two bases: (a) on the effect on potassium/sodium ratio, which gives a more exact estimate of dosage (Table 1: 95 per cent confidence limits in parentheses) and minimizes error due to nonspecific variations in sodium; and (b) on the effect on sodium output, which is reduced by aldosterone, desoxycorticosterone and a few closely related compounds, but not by cortisone.

Five normal men and women on unrestricted diets showed sodium-retaining activity averaging 86 μg. of DCA-equivalent per twenty-four hours. Slightly smaller amounts were recovered from the urines of 4 normal children (Table 1).

No sodium-retaining corticoid was found in the urine of a patient with Addison's disease (probably due to adrenocortical atrophy) and of 2 patients after bilateral adrenalectomy. Since the urinary fractions caused a slight increase in the output of sodium, the results are the opposite of those following administration of DCA and give an estimate of DCA dosage less than zero. These findings favor the supposition that the sodium-retaining corticoid arises in the adrenal cortex.

In 2 patients with panhypopituitarism, the output of sodium-retaining corticoid was comparable to the levels found in normal men. These 2 patients suffered from expanding pituitary tumors, which had been temporarily arrested by irradiation or surgery. Both patients showed asthenia, impotence, atrophy of the testes, and moderate loss of weight, together with a low basal metabolism. They had received testosterone, cortisone and desiccated thyroid but medication was withheld during the present studies. One was a 52-year-old man, whose gonadotropin excretion was less than 5 m.u.u. per day, and 17-ketosteroid excretion 3.4 mg. per day. Sodium-retaining corticoid was in the normal range. It may be of interest that this patient had not had clinical manifestations of adrenocortical insufficiency. The other patient, a 43-year-old man, had hypotension, with a serum sodium level of 138 mEq./L, a urinary 17-ketosteroid excretion of 4.9 mg. per day, impaired water diuresis (Robinson-Power-Kepler test), a low output of neutral reducing lipid, and an increase in circulating eosinophils after a single intramuscular injection of corticotropin. Sodium-retaining corticoid was not significantly reduced, despite clinical and chemical evidence of a deficiency of hydrocortisone-like hormones and of 17-ketosteroid output.

It is apparent that a relatively normal output of sodium-retaining corticoid can be maintained, even though the hypopituitarism results in deficient adrenocortical output of hydrocortisone-like hormones and 17-ketosteroids. This observation is in accord with Swann's conclusion (11) that the sodium-regulating function of the adrenal cortex is largely independent of the pituitary, and with other indirect anatomic and clinical evidence (12, 13).

The average output of sodium-retaining corticoid in 4 normal children

was approximately two thirds of the adult average. In children, as in the adult with pituitary failure, the output of sodium-retaining corticoid appears to be independent of those adrenocortical secretions which are excreted as 17-ketosteroids.

Acknowledgment

The authors wish to thank Dr. Francis S. Greenspan and Mr. William Lew for making available their observations on the patients with pituitary failure; and Miss Anne Dowdy, Mrs. J. Harvey, and Mr. Way Lew, for technical assistance.

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Questions and Answers

(Questions submitted at the 1953 Postgraduate Assembly of the Endocrine Society and the University of Minnesota Medical School)

Answers by R. W. Rawson and O. H. Pearson, Memorial Center for Cancer and Allied Diseases, New York, N. Y.

QUESTION: What is the current feeling concerning the relationship between growth hormone and cancer growth?

ANSWER: Evidence for growth hormone having a significant effect on the growth of certain tumors has been reported by Moon and associates. After administration of growth hormone over a period of many months, they observed in their animals reticulum-cell sarcoma-like lesions in the lungs, tumors of the adrenals which seemed to arise in the adrenal medullas, ovarian tumors which had the structure of thecomas or granulosa-cell tumors, and benign breast tumors. In man, the only evidence is an occasional observation of one or more tumors in patients with acromegaly. In this institution, we have seen one patient who had acromegaly, multiple lipomata, a benign lesion in the skeleton and a papillary carcinoma of the thyroid. At the Massachusetts General Hospital, we were very much impressed by an acromegalic patient who had a carcinoma of the lip which was of many years' duration; although the lesion measured about 1 cm. in diameter, there were no cervical nodes. This patient's history was that of acromegaly which had existed for many years and apparently burned out, followed by panhypopituitarism just about the time that the lesion on the lip was first noted. At the New York Hospital Dr. Rall has been studying the records of cases of acromegaly to determine whether or not there is an increased incidence of carcinoma in these people as compared to the rest of the population. It has been reported by Joslin *et al.* that the incidence of cancer in diabetes is greater than in nondiabetics and it has also been reported from the State Cancer Hospital in Missouri that the incidence of diabetes and of diabetic glucose tolerance curves in patients with endometrial cancer is considerably higher than in the normal population.

The experimental and inductive evidence in humans is strongly suggestive that growth hormone may promote the growth of cancer; but it appears, at least in the animals, that the carcinogenic action of growth hormone must have the stimulus of some other pituitary hormone, or the hormone of a pituitary target-organ for the development of such tumors.

QUESTION: There is a plethora of evidence—pathologic and statistical—that prolonged treatment with estrogens postmenopausally is related to a significantly higher incidence of carcinoma of the endometrium than in the general population. Do you reject this and suggest prolonged use of estrogens?

ANSWER: We are not aware of the plethora of evidence that prolonged treatment with estrogen in postmenopausal women is related to a significantly higher incidence of endometrial cancer. There have been isolated reports of patients in whom uterine cancer de-

veloped after prolonged treatment with estrogen, but it seems that a large percentage of postmenopausal women in this country are receiving estrogens. On the other hand, the incidence of cancer of the endometrium in women with granulosa-cell tumors or thecomas is certainly significant. The level of circulating estrogen has not been established in such patients. Indeed, this question awaits the type of studies that Dr. D. West is carrying on here at the present time. We neither reject nor suggest prolonged use of estrogens When estrogen therapy is indicated by severe menopausal symptoms, very atrophic mucous membranes of the mouth or the vagina, or troublesome osteoporosis, it should be administered in doses adequate to control the symptoms. However, we do advocate that the administration of estrogens be intermittent. We usually give the hormone daily for twenty days and have a rest period between each 20-day period of treatment.

QUESTION: In a young woman with breast cancer, would you create an artificial menopause by castration and then use stilbestrol for further effect on the cancer?

ANSWER: We would create an artificial menopause by castration in a young woman with metastatic breast cancer. About 50 per cent of patients will obtain objective benefit. The benefit is due to removal of estrogen; thus, stilbestrol should not be given to these patients.

QUESTION: In cancer of the breast with bone metastases, is it ever practical to give stilbestrol and testosterone, or to alternate them. How should these be given?

ANSWER: The studies made in this hospital by Dr. Pearson would indicate that there are certain tumors which are estrogen-dependent. Such tumors apparently are stimulated by the administration of even physiologic doses of estrogens and are often inhibited by the administration of an anti-estrogen such as testosterone. Thus, we should be reluctant to alternate stilbestrol and testosterone but should make every effort to determine the sensitivity of such tumors to one agent or the other and then administer that agent as long as it exerts any growth-inhibiting influence on the tumor.

QUESTION: In cases of breast cancer, what has been the response of brain metastases to androgen or estrogen therapy?

ANSWER: The response of brain metastases from breast cancer to androgens or estrogens in our own group has not been well established. There has been reluctance to administer either of these hormones, as they may promote the storage of water in the patient having cerebral disease. These are, however, a few isolated instances of tumors which have responded to androgens.

Answers by George W. Thorn, Peter Bent Brigham Hospital, Boston, Mass.

QUESTION: What is the evidence that there is benefit from treating multiple sclerosis with ACTH or cortisone?

ANSWER: The occasional presumed beneficial result of corticoid treatment of multiple sclerosis is difficult to evaluate, since irregular spontaneous remissions and exacerbations mark the course of the disease (1). A report of 10 cases from this laboratory (2) demonstrated no clinical improvement deriving from short-term intravenous ACTH administration. In all cases, indices of adrenocortical response were normal, indicating that

adrenal hypofunction is not a factor in multiple sclerosis. Consequently, corticoid therapy should be maintained at pharmacologic levels for prolonged periods, and several adequately controlled large series of cases are required before a definitive answer can be given to the question of possible benefit in multiple sclerosis from adrenocortical hormone therapy. Objective appraisal of such series requires concurrent placebo administration, so directed that neither patients nor examining physicians know when adrenocortical hormones are being dispensed to a given subject.

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QUESTION: Should the daily dosage of cortisone be divided? Is there any difference between parenteral cortisone and oral cortisone in this respect?

ANSWER: The rate of onset and duration of the action of cortisone acetate have been assessed by eosinophil depression (1), plasma 17-hydroxycorticoid concentration (2), and effects on electrolyte metabolism.

When cortisone is given by mouth, the maximum fall of the eosinophil count is found at four hours. With doses of 50 mg., the counts return to normal within eight to twelve hours, and with 100 mg., within twelve to twenty-four hours. After oral doses of 200 mg., plasma 17-hydroxycorticoid concentrations reach their maximum in one hour and return to normal in eight to twelve hours. Changes in electrolyte excretion can be recognized shortly after the administration of 50 and 100 mg. doses, but pass off within a few hours of stopping treatment.

Judged by these criteria, cortisone acetate by intramuscular injection is much slower in onset of action and less effective than when given orally. Doses of 100 mg. or more are required to produce any appreciable depression of the eosinophil count; moreover, the fall is not as great and does not reach its lowest level until twenty-four hours. Changes in electrolyte excretion are also less striking; they reach their maximum in four days and may persist for a further four days. Even after doses of 200 mg. intramuscularly, little change may be found in the plasma concentration of 17-hydroxycorticoids. It is interesting to observe, however, that in the treatment of congenital adrenal hyperplasia, intramuscular injections are more efficient than similar doses given by mouth in suppressing adrenocortical activity (3).

Thus, with small oral doses, for the maximum therapeutic effect, six-hourly administration is best. In the higher dosage ranges, maximum effects may be obtained with twelve-hourly oral administration. There is no advantage in giving intramuscular cortisone more often than once daily.

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The Endocrine Society

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

Those wishing to present papers, (time limited to ten minutes) should send four copies of the title and abstract to the Vice-President, Dr. E. B. Astwood, 30 Bennet Street, Boston 11, Massachusetts, not later than *February 1, 1955*. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. Neither footnotes nor acknowledgments to sponsors can be published. References, if used, should be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading should be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institute of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, as there will be no opportunity for proof reading by authors.
5. Abstracts not conforming to the foregoing regulations will be returned to the authors.

THE 1955 AWARDS AND FELLOWSHIPS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be

divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

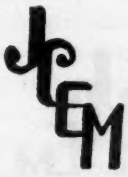
Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

Nominations

Nominations for the Medal of The Endocrine Society, the Ciba Award, and the Ayerst, McKenna and Harrison Fellowship may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the office of the Secretary, Suite 319, 1200 North Walker Street, Oklahoma City 3, Oklahoma. *Completed nominations should be returned to the Secretary not later than November 1, 1954.*

Proposals for appointment as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. *They should be submitted by November 1, 1954.* The Awards Committee will meet in November and notice of awards to successful applicants will be made not later than December 1, 1954.



The American Goiter Association

THE 1955 ANNUAL MEETING

The next annual meeting of the American Goiter Association will be held in Oklahoma City, Oklahoma, at the Skirvin Hotel, April 28, 29 and 30, 1955.

THE AMERICAN GERIATRICS SOCIETY

The American Geriatrics Society will give a *Graduate Symposium on Geriatric Medicine* at the Roosevelt Hotel, New York City, November 11 and 12, 1954.

This is a service provided by the Society for its Fellows and all members of the medical profession.

Distinguished specialists from our leading medical schools and teaching hospitals will conduct the symposium.

We cordially invite all interested physicians to attend this important symposium.

There will be no registration fee.

The Roosevelt Hotel in New York has set aside a number of rooms to take care of physicians attending this symposium. Those who plan to attend should write to Mr. A. G. Schellenberg, Reservation Manager, Hotel Roosevelt, Madison Avenue at 45th Street, New York 17, New York. Mention that you will attend the symposium and Mr. Schellenberg will be glad to take care of your needs.

For further information, write the Secretary, Dr. Malford W. Thewlis, 25 Mechanic Street, Wakefield, R. I.